

# Planting Seeds of Hope

New drug production method could provide affordable, safe treatment for stroke victims

**Dr Kevin Yueju Wang** and his team, from Northeastern State University, focus their research on the sustainable, low-cost production of drugs to treat stroke victims. They are pioneering the use of transgenic plants to produce drugs that dissolve stroke-causing blood clots.



## **Why is it preferable to use plants (rather than mammalian cells) to produce drugs?**

Plants can be grown and maintained at much lower cost than bacteria, yeast or mammalian cells. This means initial studies can be done with a lower financial investment. Additionally, once created, the source (leaves or seeds) is potentially unlimited. They are also preferable to other types of cell in terms of cost, safety and the speed of production. They are able to produce the complex proteins that are required, including antibodies. Unlike mammalian cells, there is also no risk that the drug will become contaminated by animal pathogens, for example viruses. This is a huge boost for the safety of these drugs. *Read more:* <http://www.ncbi.nlm.nih.gov/pubmed/26633378>

## **Can you give a brief overview of how these plants are genetically modified?**

We identified potential genes (DSPA $\alpha$ 1 and tPA) suitable for plant-based protein development. Both genes were synthesised and cloned in a plant gene expression vector. Both genes were driven by seed-specific promoters, which target expressed proteins only in seeds. The tobacco leaf discs will be transformed using the natural genetic engineer, *Agrobacterium tumefaciens*. The shoot grown from a single cell containing foreign genes will be screened by molecular techniques, and then selected for producing seeds. Proteins from seeds will be extracted and purified. They will then be used to dissolve fibrin and blood clots.

## **What are the challenges of using transgenic plants to produce drugs?**

The field of using transgenic plants for pharmaceutical drugs is relatively new and, as such, there are not many existing protocols for us to follow. Each plant species is made up of unique sets of proteins and metabolites so the purification process is also unique for each species. The complexity of the plant make-up means that designing a purification

method (to isolate the proteins produced by the transgenic plant) is very complex. In addition, crops grown in fields (like wheat or rice) raise cross-contamination concerns: the pollen from the transgenic plant may contaminate normal plants that are being grown nearby. Currently, the use of food crops for the production of recombinant pharmaceutical compounds (made using transgenic plants) is restricted.

Tobacco is an excellent candidate for pharmaceutical production. It is not a food crop and has a simple gene transfer system. The plants can be produced in just six months and both the leaves and the seeds can be used for production. The systems for ensuring biosafety and the processes for purification have both been established for tobacco.

*Read more:* <http://www.ncbi.nlm.nih.gov/pubmed/26633378>

## **Are there any other natural anticoagulant protein sources that can be used to treat stroke?**

There are currently no effective anticoagulant proteins used to treat stroke. Some thrombolytic agents are available to prevent stroke. These include, among others: streptokinase, secreted by several species of streptococci; urokinase, found naturally in humans, especially in urine; nattokinase from fermented soybeans, a dish known as natto; and lumbrokinase from earthworms. We are working on using plants to produce these anticoagulant proteins.

## **How do you plan to develop your research in the future?**

For current tPA or DSPA $\alpha$ 1, we are refining the purification process. We expect to produce proteins with 99.9% purity for our pre-clinical safety test. In the future, we will conduct clinical trials and put the proteins into clinic usage. We will discover more candidate genes that meet a commercial need. We will develop a good production system and build a bridge between basic research and its commercial applications.

Strokes are a severe and life-changing disease. Not only do they cause 15 million deaths per year worldwide, they also have potential long-term consequences for survivors, including impaired vision and mobility, dementia and depression. Unfortunately, the incidence of stroke is increasing as the global population ages. In order to combat this devastating disease, the development of cheap, efficient drugs is vital and Dr Wang's team is currently focussed on achieving this goal.

#### CAUSES OF STROKE

During an acute ischemic stroke (85% of cases), arterial blood clots reduce the flow of blood to the brain. Brain cells, starved of oxygen and essential nutrients, rapidly deteriorate and die.

There are two key components of a blood clot: aggregated platelets and a structural mesh of the protein fibrin. Currently, acute ischemic strokes are treated using a medication called alteplase, otherwise known as tissue plasminogen activator (tPA). This enzyme breaks down fibrin, destroying the blood clot and restoring blood flow to the brain.

Currently, alteplase is the only Food and Drug Administration (FDA) approved drug for the treatment of acute ischemic stroke. However, there is much debate regarding the effectiveness of alteplase – Dr Wang draws attention to its high level of side-effects in particular. Research has shown that alteplase must be taken within 3-4.5 hours of a stroke occurring. Any delay increases the risk of severe side effects such as neurotoxicity, brain damage or potentially life-threatening haemorrhage. Stroke victims frequently do not seek emergency treatment within this opportunity window meaning that alteplase cannot be used as a treatment for the vast majority of stroke victims: it is currently only administered to around 2% of patients.

#### VAMPIRE BAT SALIVA – A BETTER DRUG?

The natural world has often provided inspiration to those looking to solve human problems and, after much research, scientists discovered that the proteins found in vampire bat saliva could provide a significantly more effective and safer therapeutic option for treating stroke than alteplase.

Vampire bats prey on other mammals, feeding on their victim's blood. To enable continued feeding, vampire bats have



**In comparison to mammalian-based systems, the use of transgenic plants is significantly cheaper and safer, as there is a reduced risk of contaminating the drug with pathogens**

evolved mechanisms to delay clotting and so prolong bleeding. For example, their saliva contains anticoagulants, including the proteins, *Desmodus rotundus* (vampire bat) salivary plasminogen activators, or DSPAs. Although there are several types of DSPA, the majority of studies focus on DSPA $\alpha$ 1 for treating ischemic stroke. Dr Wang and his team have decided to carry out their research using both DSPA $\alpha$ 1 and DSPA $\alpha$ 2 – a little studied DSPA that the team's preliminary work shows also attacks clots. DSPA $\alpha$ 1 can bind more tightly to fibrin than alteplase, increasing its efficacy. Additionally, DSPA $\alpha$ 1 can be administered up to 9 hours after a stroke, greatly increasing the number of people who can be treated. It is also relatively safe: unlike alteplase, DSPA $\alpha$ 1 does not act on certain brain cell receptors, meaning there is less risk of brain damage, and it has not been seen to cause overbleeding.

However, further testing, such as animal trials, is required before DSPA $\alpha$ 1 is considered safe for therapeutic use.

#### THE CHALLENGES OF CURRENT DRUG PRODUCTION

Currently, mammal cells are used to produce alteplase on an industrial scale. If DSPA $\alpha$ 1 was in wide-spread use today, it would also be produced using these methods. These mammal cells are used because they are like minuscule factories – capable of constantly producing new proteins. By manipulating these cells using 'recombinant technology', scientists can programme the cells to produce the desired alteplase protein.

'Recombination' involves combining DNA from different organisms to produce a new protein. In the case of drug production, the section of DNA that codes for alteplase is

inserted into the mammal cell genome. This mammal cell is now 'transgenic'. The alteplase protein (tPA) is therefore expressed in the mammal cell. After filtration and purification of the tPA from the other cellular components, it is then ready to be developed into a drug.

However, using mammal cells as a way of producing drugs on a large scale has several major disadvantages. For example, production is very expensive – in 2014 a dose of 100mg of tPA cost around US \$6,400! Additionally, this technology is low-yielding and the risk of cell culture contamination (for example, through viruses that use mammal cells as host cells) is high. Dr Wang and his team have developed an innovative method to overcome these challenges by using plants to produce both tPA and DSPA $\alpha$ 1.

#### PLANTS AS DRUG FACTORIES

Scientists and drug companies are viewing plants in a new light – as effective and sustainable pharmaceutical factories. For example, by using transgenics, the tobacco plant, which, paradoxically, is responsible for causing many deaths worldwide, has been used as a tool to produce drugs that fight deadly diseases, including malaria.

Inspired by this research, Dr Wang has developed tobacco plants to produce seeds

that contain DSPA or tPA. In a similar way to mammalian-based production systems, Dr Wang used 'recombination technology'. The team combined the DNA of DSPA with a 'seed-specific promoter'. This means that the DSPA is only made in the seed and not in any other part of the tobacco plant. These plants are then grown and cultivated in a greenhouse, the seeds are collected and the desired protein is isolated.

#### KEY ADVANTAGES

There are several reasons why seeds (instead of other parts of the plant) were selected as the drug-producing site. Firstly, there is a reduced risk of protein degradation in the seed compared to other locations such as the leaves. This means that there is less chance that the drug will be broken down before it is harvested. Additionally, seeds produce unlimited generations of drug-producing tobacco plants, so the potential yield is very high. Seed can produce recombinant protein at high yield and maintain protein stably at room temperature up to several years. Most seeds can be subjected to a surface 'sterilisation' without damage to the proteins.

In comparison to mammalian-based systems, the use of transgenic plants is significantly cheaper and safer, as there is a reduced risk of contaminating the drug with potentially dangerous animal viruses and other pathogens. Furthermore, due to the high yield of plant-based systems, the cost of the resultant DSPA $\alpha$ 1 or alteplase (tPA) is much cheaper – an estimated cost of around \$450 per dose for either treatment.

#### WHAT DOES THE FUTURE HOLD?

Even though tPA has side effects, it is still the only drug approved for acute ischemic stroke. We can use plants as an alternative production system to produce tPA at low cost. In order to fight stroke safely and effectively, it is also imperative that we develop better drugs than tPA. Research has shown that DSPA $\alpha$ 1 from vampire bat saliva meets these demands as a safe alternative that can be used to treat a larger number of patients. In addition, Dr Wang has shown that, by using transgenic plants rather than mammalian-based systems, we can produce these drugs both cheaply and efficiently with a high, sustainable yield. The hope for the future of this research is that DSPA $\alpha$ 1 production by transgenic plants will be commercialised, enabling significantly more people to be saved from the devastating consequences of stroke.

## Detail

#### RESEARCH OBJECTIVES

Dr Kevin Yueju Wang targets recombinant proteins in plant seeds for the treatment of stroke patients. Seed platforms can be used to produce a variety of active, safe, and inexpensive therapeutic proteins.

#### FUNDING

- NIH – National Institute of Neurological Disorders and Stroke
- NIH – National Institute of General Medical Sciences

#### COLLABORATORS

- Hangzhou Funiu Pharmaceutical Biotech Ltd.
- Jiayi (Joy) Cheng

#### BIO

Dr Kevin Yueju Wang is an Associate Professor at the Northeastern State University. His team are applying transgenic plants for production of human therapeutic proteins on a large scale at low cost.

#### CONTACT

Department of Natural Sciences  
Gregg Wadley College of Science & Health Professions  
Northeastern State University  
3100 E. New Orleans  
Broken Arrow, OK 74014, U.S.A.

**E:** wang03@nsuok.edu

**T:** +1 (918) 449-6479

**W:** <https://www.nsuok.edu/directory/profile/wang03.aspx>

#### FOR MORE INFORMATION VISIT

- <http://www.kjrh.com/news/local-news/northeastern-state-univ-research-finds-affordable-alternative-to-combat-stroke-effects-with-bat-dna>
- <https://www.youtube.com/watch?v=HMAAdVAAJ33c>
- <https://www.google.com/patents/WO2016118732A1?cl=en>