Anti-Parasite Drugs for Neglected Chagas Disease Are in the Works

July 21 2010, By Jeffrey Norris

The kissing bug, Rhodinus prolixus, spreads the parasite that causes Chagas disease. Credit: Erwin Huebner, University of Manitoba

Larissa Podust, PhD, is working on a new drug for a neglected scourge, a chronic parasitic infection called Chagas disease. The parasite is carried and spread by the kissing bug.

The kissing bug is a beetle that “kisses” humans on the lips or face - but the kiss is really a blood-sucking, infectious bite. What’s worse is that the little vampire then defecates on the skin. The parasite is in the waste. Scratching the wound provides an opportunity for the parasite, Trypanosoma cruzi, to get into the bloodstream.

In the United States, Chagas disease remains rare. But at least a few

hundred thousand Latin American immigrants are believed to have been infected earlier in their native countries. A handful of people may have acquired the disease in the United States in recent years.

It may be below the radar in the United States, but worldwide, Chagas disease chronically afflicts and wears down roughly 10 million people. In those who have a chronic infection, the parasite can enter and destroy heart muscle cells, eventually leading to death due to heart failure.

There are no drugs on the market that specifically target the disease. "The only approved drugs have been in use for 50 years, and both are extremely toxic," Podust says. But Podust and other lab leaders at UCSF's Sandler Center for Drug Discovery aim to change this state of affairs.

"To wait 50 years between drugs is unacceptable," Podust says.

Sandler Center Director James McKerrow, MD, PhD, is looking forward to the US Food and Drug Administration granting investigational new drug (IND) status - a step closer to clinical trials - for a UCSF-developed drug candidate called K777. K777 targets a parasite enzyme called cruzain. Podust is working on the next wave of new drugs, targeting a different protein, called CYP51.

CYP51 might at first seem an odd choice for a target. The enzyme is not unique to T. cruzi. It is found in microbes, fungi, animals and humans. But the DNA encoding the enzyme is significantly different among organisms, as are the enzymes made according to those genetic instructions. T. cruzi needs CYP51 to make steroids for cell membranes.

Chagas Disease Has Been Neglected

Chagas disease is regarded as a neglected disease because it mainly

strikes poor people living in rural areas of Latin America, and drug companies have not viewed targeting T. cruzi as a moneymaking enterprise.

But from a public health perspective, in countries such as Brazil, a focus on T. cruzi would be right on the money. In fact, for years, the life cycle of T. cruzi was depicted on the 10,000 cruzado note - with illustrations of the single-celled parasite with its whip-like tail and of the transmission of the disease via the kissing bug's droppings.

Two drugs originally developed by pharmaceutical companies to treat fungal infections - posaconazole, branded as Noxafil by Schering-Plough, and ravuconazole - are now going to be evaluated in clinical trials for Chagas disease.

However, development of the two drugs was not the result of deliberately targeting key proteins in pathogens, Podust says, even though they do inhibit CYP51. Through random screening of chemical libraries, they were shown to be effective in fighting fungal infections.

Chagas Disease Drug Target Proves to Be Prize-Winning

Podust recently won an annual competition among students, postdoctoral fellows and faculty from Bay Area and San Diego universities that was organized by the Wheeler Center for Emerging and Neglected Diseases (CEND) at UC Berkeley. Podust won the prize for work demonstrating that T. cruzi CYP51 was the best drug target in a neglected disease among those submitted for the contest.

According to Tom Alber, PhD, CEND faculty director, "The big motivation for the contest is to educate the infectious disease research

community about the characteristics of a great drug target." Three finalists, including Podust, made their pitch from the podium. During her talk, Podust "made a powerful case" that impressed the judges from Bay Area investment and biotech companies, Alber says.

First, Podust selected a target that is absolutely needed by the parasite. She then developed a high-throughput test, an assay to screen hundreds of thousands of chemicals to see which can interfere with CYP51 enzyme activity.

Podust identified tightly binding inhibitor molecules through this screening, and began using one as a scaffold, modifying it with other small chemical groups to further improve the specificity of drug prototypes for the targeted enzyme.

The best molecule built from this scaffold killed T. cruzi in laboratory cell cultures. More recently, Podust's lab group has shown that this T. cruzi CYP51 inhibitor eradicates the parasite in mice.

"Among the 10 inhibitors which we originally investigated, we found one that is nontoxic, that binds well to the target and that cures the disease in mice," Podust says.

Alber says that the contest judges, David Mack, PhD, and Adam Tomasi, PhD, from Alta Partners and Gideon Bollag, PhD, from Plexxikon Inc., remarked on the importance of having more than one scaffold to build drugs from because many can be expected to fail. Podust aims to build new inhibitors upon the other tightly binding scaffolds she has identified during high-throughput screening.

"All drugs have side effects, but we are trying to make rationally designed drugs that would be more efficient and that would be less cross-reactive with the human enzyme," she says.

Identifying the Three-Dimensional Structures of Drugs and Drug Targets

Podust, who for the past 10 years has been working on drug targets as a university scientist, favors drug design based on an understanding of the target's shape. Her approach has been to precisely determine the three-dimensional structure of an enzyme that the parasite needs to survive. Enzymes bind to other molecules to chemically alter them, using chemical energy to drive crucial biochemical processes.

The part of the enzyme structure that interacts with and changes the molecule to which it binds is called the active site. In targeted drug development, researchers often aim to make molecules - drug prototypes - that interfere with the enzyme's ability to bind to or transform the natural molecule.

Podust's main expertise is in X-ray crystallography, a technique for determining protein structure by purifying the protein and getting it to form crystals. X-ray crystallographers bounce X-rays off the regularly repeating protein structures. The patterns formed by the X-rays as they are refracted off the crystal and onto a detector can be analyzed to determine the three-dimensional structures of proteins in atomic detail.

Podust was the first to determine the structure of T. cruzi CYP51, and recently she determined the structure of the enzyme bound to the antifungal inhibitors, shedding light on drug action and drug resistance.

Collaborators in Their Spare Time

Podust has some Sandler Center funding, and the prize for ranking first in the CEND competition included support from CEND and the UCSF Small Molecule Discovery Center (SMDC) to screen CYP51 against the

SMDC's chemical library. But much of Podust's ongoing work for the past 10 years has been done with minimal funding and in "her spare time," she says. "We are going to continue to explore active-site interactions with different molecules," Podust adds.

"This project requires a lot of different kinds of expertise," she says. Despite the dearth of funds, Podust has engaged researchers from UCSF and Scripps Research Institute to continue work. She is taking advantage of the core laboratory services at the California Institute for Quantitative Biosciences (QB3), including the SMDC, and at the Protein Crystallography Beamline at Lawrence Berkeley National Laboratory.

In addition, Podust says, "At UCSF, we have had more success working with parasites in animal models and cell cultures. It's a great place to be for this project."

Provided by University of California, San Francisco