Scientists reveal how an old drug could have a new use for treating river blindness

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Scientists at The Scripps Research Institute have discovered a potential new use for the drug closantel, currently the standard treatment for sheep and cattle infected with liver fluke. The new research suggests that the drug may be useful in combating river blindness, a tropical disease that is the world's second leading infectious cause of blindness for humans.

The study is scheduled for publication in an advance, online Early Edition of the journal Proceedings of the National Academy of Sciences (*PNAS*) during the week of February 8, 2010.

The new research shows that clostanel has the potential to inhibit the molting process of the parasite that causes the disease.

"We think this finding holds terrific potential for the treatment of river blindness, one of 13 recognized neglected tropical diseases," says Scripps Research postdoctoral fellow Christian Gloeckner, the first author of the study.

Professor Kim Janda, who is director of the Worm Institute for Research and Medicine, Ely R. Callaway Chair in Chemistry, and member of The Skaggs Institute for Chemical Biology at Scripps Research, adds that there is an urgency to fighting the infection that leads to river blindness, which is also known as onchocerciasis. Despite several eradication efforts, the disease affects more than 37 million people in Africa, Central and South America, and Yemen.

"Victims of onchocerciasis suffer severe skin lesions, musculoskeletal pain, and various stages of blindness," says Janda, adding that patients also experience decreased body mass index, decreased work productivity, and social stigmatization.

River blindness is caused by thread-like filarial nematode worms, Onchocerca volvulus, which are transmitted among humans through the bite of a black fly. The nematodes then multiply and spread throughout the body. When they die, they cause a strong immune system response that can destroy surrounding tissue, including that of the eye. Currently, the only drug available for mass treatment of river blindness is ivermectin, and it now appears that resistance to that drug is emerging.

This creates a critical need to identify new drug targets and agents that can effectively treat the disease.

Building on Recent Discoveries

The current study builds on recent research that has implicated chitin metabolism in the larval development of the parasite *O. volvulus*.

Chitin is the protective outer covering that forms part of *O. volvulus*'s outer cuticle. While knowledge of chitin biosynthesis in nematodes is limited, scientists do know that two classes of enzymes are critical for maintenance of the pathway—chitin synthases and chitinases, digestive enzymes that break down glycosidic bonds in chitin. The dynamic synthesis and degradation of chitin by these enzymes is a prerequisite for the organism's development and therefore a potential drug target.

Researchers in the field had recently identified and characterized one interesting chitinase from *O. volvulus*, OvCHT1. Although OvCHT1's exact metabolic role is not known, it was found to be expressed only in the infective L3 larvae and to have potential involvement in host

transmission, molting, and important developmental processes in the parasite. Immunoelectron microscopy analysis detected chitinase in the pharyngeal glands of *O. volvulus*, structures that may contain a wide variety of proteins essential for the remodeling processes during molting and the shedding of the old cuticle.

"Therefore, we focused on these enzymes," Gloeckner says, "and reasoned that inhibiting them may eliminate onchocerciasis."

To test these enzyme candidates, Gloeckner, Janda and their colleagues used the Johns Hopkins clinical compound library—which contains 1,514 compounds, of which 1,082 are U.S. Food and Drug Administration (FDA)-approved drugs and 432 are foreign-approved drugs—to screen for active compounds.

"We were looking for a molecule that had a dramatic effect on chitanase specific to *O. volvulus*," Gloeckner explains. "The chitinase's enzymatic activity was monitored by a fluorescent signal. A library member was scored as a "hit" when a decrease in the signal was observed. Simply stated when a huge decrease in the signal was observed, the enzyme was essentially "knocked-out."

The screening efforts identified four known drugs namely levfloxacin, lomefloxacin, dexketoprofen, and closantel. Of these, only closantel was found to exhibit potent enough inhibition to warrant further investigation.

Cross-Country Collaboration

The next step was to find out if closantel would work in vivo, in the larvae of *O. volvulus*.

"The molting process is considered a potential new target for

chemotherapy against onchocerciasis," Gloeckner explains. "And since chitinases may play a key role in molting, we wanted to determine the effect of closantel on this process." Specifically, the researchers were interested in how clostanel would disrupt molting from the L3 to L4 stage of the larvae, a critical step that occurs within the human host.

That's when the Scripps Research team enlisted the help of Sara Lustigman's laboratory at the Lindsley F. Kimball Research Institute at the New York Blood Center. Lustigman's team cultured L3-stage larvae in the presence of increasing concentrations of closantel, and the number of larvae was determined on day six. The results? Closantel completely prevented molting from the L3 to L4 stage.

Gloeckner was excited by this finding. "Based on its specificity, potency, and ease of synthesis, closantel or one its analogues might represent a promising alternative or adjunct therapy in combination with ivermectin for the treatment of onchocerciasis," he says.

Gloeckner adds that, based on this strong evidence of efficacy, he would like to take closantel into experiments with animal models.

In addition to Gloeckner, Janda, and Lustigman, authors of the article, "Repositioning of an old drug for the neglected tropical disease Onchocerciasis," include Amanda L. Garner, Lisa Eubanks, and Gunnar Kaufmann of Scripps Research, Fana Mersha of New England Biolabs, and Yelena Oksov and Nancy Tricoche of the New York Blood Center.

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