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Scientists find new drug candidates for set of protein-folding diseases

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Collaborating researchers at Stanford University and The Scripps Research Institute have identified chemical compounds that show promise as potential therapeutics for a set of medical conditions caused by the abnormal clumping together of a protein known as transthyretin (TTR).

The compounds, which prevent the abnormal aggregation of the TTR protein, work by holding the protein together in its functional form. These compounds have the potential one day to help the hundreds of thousands of people who have TTR-related amyloid diseases or are at risk for them, and may have advantages over other TTR-stabilizing drugs, which are currently in clinical trials.

"These new compounds have structures that make them very effective at stabilizing TTR in its stable native tetrameric form in laboratory tests, and they also seem nontoxic in cell culture," said Stephen Connelly, a senior research associate in the Scripps Research laboratory of Professor Ian Wilson.

Connelly, who determined the molecular structures of these TTR-stabilizing compounds, is a co-lead-author of the report, which appears in the current issue of *Science Translational Medicine*. The other lead author is Mamoun M. Alhamadsheh, who at the time of the study was a postdoctoral researcher in the laboratory of Isabella Graef, an assistant professor of pathology at Stanford University.

Defenses against Abnormal Forms

TTR proteins normally don't work alone. Single "monomeric" copies come together in pairs, and then in pairs of these pairs, to form four-protein structures known as "tetramers." Secreted by the liver into the bloodstream, TTR tetramers work as transporters of the hormone thyroxine and also bind the holo retinal binding protein. In the hustle and bustle of the bloodstream, however, TTR tetramers often come apart, and when that happens, the naturally sticky individual TTR proteins may start to re-form abnormally, into toxic fibril-shaped aggregates known as amyloids.

"It's well known that the body's normal defenses against amyloids decline with aging," said Graef. Apparently for that reason, TTR amyloids are found at autopsy in the heart and other organs of 10 to 15 percent of people over 65, although they almost never accumulate in young people.

TTR amyloids don't always cause symptoms, but in many cases they do impair functions or hasten age-related degeneration. Inherited mutations of the TTR gene can cause earlier-onset TTR amyloid conditions, including familial amyloid cardiomyopathy, which often causes heart failure and is particularly common in West Africans and African-Americans.

First-generation drug candidates for preventing TTR amyloid formation have been developed, and two are already in clinical trials. But most of these have chemical similarities to non-steroidal anti-inflammatory drugs (NSAIDs). As such, they have potentially harmful side effects—including to the heart and kidney—that would make them less than ideal for long-term use, especially in patients with compromised heart function. Graef and her colleagues therefore developed an innovative and novel test to screen a library of compounds for those that

would bind and stabilize TTR but otherwise would not have NSAID-like effects.

They found 33 powerful TTR stabilizers with their screening system. "Many of these were novel chemical entities with no previously known biological targets," Graef said. After selecting the most potent of the compounds—some of which seemed even more potent than those in clinical trials—she and her team used further lab tests to confirm the compounds' effectiveness at preventing amyloid formation by normal and mutant forms of TTR. Preliminary tests of the compounds' toxicity also showed that they did not appear to harm normal cells.

Strengthening Weaker Joints

Often when scientists find a promising drug candidate in initial screens, they try to determine the precise molecular shape it makes in conjunction with its target. With this information, they can modify it chemically, for example to make it bind to the target more tightly and exclusively. Thus Graef brought four of her best TTR-stabilizing compounds to Scripps Research for structural analysis. "We have one of the world's largest and most advanced facilities for determining protein structures, and we've done this for almost 30 TTR-drug complexes to date" Connelly said.

Using cutting-edge X-ray crystallography techniques, Connelly soon solved the structures of the four compounds as they bound to TTR. "Despite their diverse chemical differences, all four turned out to bind to the TTR tetramer in ways that span and thereby strengthen its weaker joints, stabilizing the healthy tetrameric form," said Connelly.

Graef and her colleagues at Stanford now are trying to gather more data on the effectiveness and safety of the more promising compounds. "Together with physicians from a cardiovascular clinic here at Stanford,

we're investigating whether these compounds can stabilize, in a solution of blood serum, the TTR proteins of patients with a common familial amyloid cardiomyopathy mutation," she said. "If it can, then hopefully the pharmaceutical industry will want to develop it from there."

More information: "Potent Kinetic Stabilizers That Prevent Transthyretin-Mediated Cardiomyocyte Proteotoxicity," stm.sciencemag.org/content/3/97/97ra81.abstract

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