Scientists identify potential new class of drugs to combat hepatitis C

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Stanford University School of Medicine scientists have discovered a novel class of compounds that, in experiments in vitro, inhibit replication of the virus responsible for hepatitis C. If these compounds prove effective in infected humans as well, they may dramatically accelerate efforts to confront this virus's propensity to rapidly acquire drug resistance, while possibly skirting some of the troubling side effects common among therapies in current use and in late-stage development.

"Hepatitis C virus, or HCV, is a huge problem," said Jeffrey Glenn, MD, PhD, associate professor of gastroenterology and hepatology, and director of Stanford's Center for Hepatitis and Liver Tissue Engineering. "It infects over 150 million people worldwide, many of whom don't even know they have it. Chronic hepatitis C infection is the No. 1 cause of liver cancer and liver transplantation in the United States."

Current treatments for hepatitis C, Glenn said, are only somewhat effective and often toxic. And designing a new antiviral agent is difficult, because a virus thrives by commandeering a host cell's own essential functions.

There are many effective drugs for diseases caused by bacteria. Bacterial cells, like our own, are fully functioning units. But they differ from mammalian cells in ways that make it feasible for them to be attacked with drugs that mostly leave our own cells alone. Antibiotics, which fight bacterial infections, have revolutionized the treatment of contagious disease.

Designing a clean antiviral drug is another story. Unlike bacteria, which multiply by dividing, a virus reproduces by breaking into cells and diverting their manufacturing machinery to produce copies of itself, which eventually depart the ravaged cell to find and exploit fresh ones.

HCV is an especially tough nut to crack. Natural isolates of it can't be grown in culture as can many other viruses, which seriously impedes drug and vaccine research. (There is still no vaccine for hepatitis C.) In recent years, virologists have developed surrogate systems that substantially duplicate the HCV replication process. These systems can be used to test compounds for effectiveness against the virus.

But even when a compound shows effectiveness, HCV mutates readily. So it can rapidly acquire drug resistance. The ultimate solution, Glenn said, is probably to "attack the virus from multiple angles all at the same time with a cocktail of compounds," each targeting a different item in the virus's toolkit. "It's imperative to identify new classes of potential drugs."

Glenn is the senior author of a study, appearing online Jan. 20 in *Science Translational Medicine*, in which he and his colleagues found a brand-new class of compounds capable of disrupting the HCV replication cycle. (The study's first author is Nam-Joon Cho, a postdoctoral scholar in Glenn's laboratory.) Importantly, the identified compounds do this by interfering with a virus-initiated activity that, while critical to viral replication, doesn't ordinarily occur in uninfected cells. This, Glenn said, offers the prospect of inhibiting this activity — and stopping viral replication in its tracks — with little or no toxicity to human cells.

Animal cells are composed mainly of water and water-soluble substances, enclosed within a fatty outer membrane and segregated into distinct subcellular compartments by internal membranes. HCV replicates only in association with such membranes. While some viruses

cozy up to membranes that already exist inside living cells, HCV highjacks bits and pieces of membranes and assembles them into large clusters of tiny nested bubbles, or vesicles. The clusters are unlike anything found in a normal human cell.

Glenn and his associates identified a cylinder-shaped chunk of an HCV-encoded protein that is essential for that protein's known vesicle-aggregating activity. A synthetic version of this cylindrical section caused vesicles to aggregate into telltale clusters. The investigators then used this finding to attack the virus: they found that mutations in the synthesized segment destroyed the virus's ability to replicate.

While that insight was important, it alone did not translate into a therapy. "You can't treat a patient that way — by going in, removing all the viruses, mutating them and putting them back in," Glenn said.

Instead, the researchers set out to detect compounds that could prevent this key protein segment from working. They designed an assay consisting of hundreds of separate tiny depressions in a plastic laboratory dish, with each depression housing large numbers of individual tiny vesicles in solution. As expected, sprinkling some of the synthesized protein segment into a depression caused the vesicles inside to clump together. But laborious testing of numerous off-the-shelf compounds — a different one in each well — showed that some prevented the aggregation from happening. Two of these compounds pronouncedly impaired HCV's ability to reproduce itself in the workhorse surrogate HCV replication system.

Glenn foresees a year to 18 months of extensive preclinical and animal testing before this class of compounds can gain approval by the Food and Drug Administration to enter all-important clinical trials in humans. Because the compounds operate by disrupting a mechanism that is only needed by the virus, he said, he hopes the drugs based on them may

exhibit both viral replication-suppressing ability and a favorable toxicity profile.

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