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New approach, old drug show promise against hepatitis C, research shows

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The fight against the liver disease hepatitis C has been at something of an impasse for years, with more than 150 million people currently infected, and traditional antiviral treatments causing nasty side effects and often falling short of a cure. Using a novel technique, medical and engineering researchers at Stanford University have discovered a vulnerable step in the virus' reproduction process that in lab testing could be effectively targeted with an obsolete antihistamine.

The new research will be published in the Aug. 31 online version of *Nature Biotechnology*.

The advance involves two new discoveries. One is that a protein called NS4B is instrumental in binding some of the genetic material, or RNA, and allowing the hepatitis C virus to replicate. The other is that the former anti-itching drug clemizole hydrochloride could hinder that protein, resulting in a tenfold decrease in virus replication with no apparent harm to infected liver-like cells. Because the drug has already been used by people, it is eligible for human testing.

"We're excited about this and we're actively moving forward toward clinical trials," said virology expert Jeffrey Glenn, MD, PhD, associate professor of gastroenterology and hepatology. Glenn is one of two senior authors of the paper. The lead authors are postdoctoral scholars Shirit Einav, MD, in medicine, and Doron Gerber, PhD, in bioengineering.

One of the team's key discoveries used coin-sized microfluidic chips that

shrink tabletop biological experiments down to the tiny scale of nanoliters. The paper marks the first time that microfluidic technology has been used to discover a specific drug, said Stephen Quake, PhD, professor of bioengineering and the other senior author of the paper. In fact, the small team was able to screen more than 1,200 drug candidates and find clemizole in just two weeks, Gerber added.

"That's just an example of the power of these microfluidics automation technologies that one or two people working together can actually screen very large numbers of compounds," Quake said. "Big pharmaceutical companies have very large teams and a lot of infrastructure. We're trying to reinvent the whole process."

As director of Stanford's Center for Hepatitis and Liver Tissue Engineering, Glenn focuses his research on trying to expand the number of drug targets for the disease. After using molecular virology techniques to study the NS4B protein, he and Einav began to suspect it could be such a target.

However, like other proteins associated with cellular membranes, NS4B is difficult to purify in large quantities while retaining the protein's natural properties and functionality.

But the advantage of microfludics, Quake said, is that the volumes needed for a successful experiment are quite small, meaning that researchers can get by with very little purified, properly functioning protein. What is insufficient for a benchtop experiment is plenty in microfluidics.

"It lets us redefine the notion of success," Quake said.

Ultimately the researchers discovered that NS4B is an essential player in the virus' process of binding RNA. This is a necessary step in the virus'

replication process and, through careful observation, the team determined where it binds and how strongly. That led them to realize which kind of drug - a small-molecule compound - could block that interaction.

Even then, however, the team had to solve another problem, which is the propensity for small-molecule drugs, such as clemizole, to get absorbed into the silicone of the chip itself. Gerber said he worked around that by printing the drug onto the chip directly where the interaction with NS4B would occur. That meant the drug didn't have to move through the chip's plumbing and enough would interact with the protein.

In all, the team found 18 drugs that substantially reduced NS4B binding to its target RNA, but they focused on clemizole because it is already known to be safe in humans. Quake said several of the other compounds were also interesting starting points for developing useful medicines.

Should clemizole prove effective in human trials, Glenn said, it could become an essential component in a new class of multidrug treatments for hepatitis C. Other components could be drugs under development elsewhere that target specific enzymes in the virus. The goal is to improve on the current treatment, a combination of the general antiviral drugs interferon and ribavirin. Those only work about half the time, but have uncomfortable, flulike side effects.

"[Clemizole] does have the potential to be part of a cure, because the idea is not to use it on its own but as a cocktail component," Glenn said.

Similarly it took a cocktail of research expertise to come up with this new assault on the hepatitis C virus, Quake said.

"Neither Jeff's group nor mine could have done this on our own," Quake said. "It was enabled by both of us bringing our pieces to the table: the

questions he was asking and the technology we developed."

Source: Stanford University