In a fight to the finish, research aims knockout punch at hepatitis B

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In research published in the Jan. 24 edition of *PLOS Pathogens*, Saint Louis University investigators together with collaborators from the University of Missouri and the University of Pittsburgh report a breakthrough in the pursuit of new hepatitis B drugs that could help cure the virus. Researchers were able to measure and then block a previously unstudied enzyme to stop the virus from replicating, taking advantage of known similarities with another major pathogen, HIV.

John Tavis, Ph.D., study author and professor of molecular microbiology and immunology at SLU, says the finding may lead to drugs which, in combination with existing medications, could suppress the virus far enough to cure patients.

"Hepatitis B is the major cause of liver failure and liver cancer worldwide," Tavis said. "This would have an extremely positive effect on liver disease and liver cancer rates.

"If we can cure hepatitis B, we can eliminate the majority of liver cancer cases. This research is a step toward achieving that goal."

World health experts estimate that more than 350 million people are chronically infected with the hepatitis B virus. Several drugs are able to treat symptoms successfully, though they are not able to cure many patients. Of those infected with hepatitis B virus, up to 1.2 million die from liver failure and liver cancer each year.

A person who is infected with hepatitis B virus can have up to a billion viral copies per drop of blood. To cure a patient, a drug needs to reduce those levels to zero.

Not Quite a Cure

While existing medications are very powerful, they cannot quite deliver the knockout punch to hepatitis B. The drugs approved to treat the virus can reduce its numbers, make symptoms disappear for years and push it to the brink of extinction. But for most people, the medications can't kill the virus completely. And, as long as any virus remains, it can multiply and grow strong again.

And so, hepatitis B treatment usually spans decades, with costs of $400 to $600 a month, if patients can afford the medication. Expensive and beyond the means of many, some patients do not receive any treatment at all. As a compromise measure, some patients opt to take medication for a short time, staving off the damage the illness will cause for a few years.

A 19-Year Puzzle

Hepatitis B virus puts up a protracted fight in the lab, as well. For 19 years, Tavis has worked on a particular part of the virus's genetic puzzle, and until recently he had been, in his words, failing miserably.

The problem was a common one in the laboratory. Until scientists can measure a puzzle piece, they can't study it. And, until researchers have some small success, they don't know if they're on the right track or headed down a dead end.

This was the case for the particular enzyme Tavis believed held answers.

Stumped, he returned to the puzzle again and again over the years.

"Until you see that first glimmer, all negatives look the same," Tavis said. "One of the biggest skills in this job is knowing when to give up. It's not obvious when you are wasting time and when you are giving up too early."

In Tavis's case, his instinct served him well, and two years ago, he saw the first glimmer of the answer he was searching for.

A Virus's Tactics

"Viruses are genomic suitcases," Tavis said. "They have many tactics for invading and taking over our cells, using their own DNA as the blueprints."

In the case of hepatitis B virus, and,—in what turned out to be a lucky break, HIV, as well—the virus replicates by reverse transcription. In this process, viral DNA is converted to RNA and then converted back to DNA by two viral enzymes, both of which are vital to the virus's replication.

The first of these enzymes, a DNA polymerase, has been well studied in the lab. The five most commonly used hepatitis B drugs are able to treat (but not cure) the illness by blocking this enzyme.

The second enzyme, ribonuclease H (RNAseH) had eluded investigators in the lab. With no means to measure it, researchers hit dead ends even though they believed the enzyme was a promising target, in theory.

So, with five approved drugs targeting the first enzyme and none aimed at the second, Tavis sparred with RNAseH for nearly two decades.

Search for an Assay

Tavis was searching for a yardstick, of sorts.

Though it made sense to target RNAseH, no method existed that allowed researchers to measure the enzyme's activity. Tavis was looking for an assay, a way to tell if a substance would block the enyzme's function.

After years of work, Tavis and his research team saw the first glimmer of activity and were able to develop an assay for RNAseH, allowing him to begin to study the enzyme and try out promising theories about how to block it.

Borrowing from HIV

Because the hepatitis B and HIV viruses both use reverse transcription, the mechanism by which they copy themselves in the body's cells, hepatitis B researchers have been able to benefit from advances in HIV research. Thanks to substantial funding, HIV research has made rapid progress since the virus's discovery. Several effective drugs for HIV treatment work by targeting the reverse transcription process also work against hepatitis B virus.

Though the viruses are quite different, Tavis and his colleagues Stefan Sarafianos, Ph.D. at the University of Missouri and Michael Parniak, Ph.D., at the University of Pittsberg believed that the shared process suggested there should be some chemical similarities that could be exploited.

"Just as every car has tires and an engine, both of these viruses have pieces that serve similar functions. You can take an engine from one car and try it in the other. It might not be a perfect fit, but it should serve the

same function."

Once the assay for the RNAseH was developed, Tavis and his team were able to try out this theory.

"We found that what worked with the first enzyme worked with the second enzyme," Tavis said. "This is a proof of principle. We're on the right track."

Tavis now has a measuring tool and evidence that a number of the techniques that stopped HIV, including inhibitors of HIV RNAseH, could also inhibit the hepatitis B virus RNAseH, showing that the parallels held true. From there, Tavis and his team went on to prove that hepatitis B replication could in fact be stopped in cells with drugs that targeted the elusive second enzyme, RNAseH.

Hope on the Horizon

With these promising advances, researchers say that the search for anti-hepatitis B RNAseH drugs is now feasible and that using similar anti-HIV compounds as a guide is likely to have a high chance of success.

The research team's next step will be to study several variations of hepatitis B virus, different genotypes of the virus, to be able to measure and study the RNAseH enzyme in all forms of the virus. Current findings demonstrated success in only some genotypes. Findings from the current study suggest some promising avenues as researchers will now attempt to block RNAseH in the two most common genotypes, B and C.

In addition, researchers will aim to improve the strength and speed of the RNAseH assay for high throughput screening, a process for rapidly screening many thousands of compounds. These developments will clear

the way for full-scale antiviral drug discovery.

Investigators have reason to hope that combining a new anti-hepatitis B RNAseH drug with the existing drugs may suppress the virus far enough to cure patients with hepatitis B.

"I anticipate a new drug targeting the second enzyme would be used together with the existing drugs," Tavis said. "They jam different parts of the process.

"The drugs we have are very good drugs. They push the virus down, but they can't quite kill it. They'll still do the heavy lifting in the future, but with an additional drug I hope we'll be able to mop up the rest. Together, they may be able to do it. We don't have a big distance we need to travel to reach that point."

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