Target for potent first-strike influenza drugs identified

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(Medical Xpress) -- Scientists at St. Jude Children's Research Hospital have reported details of how certain drugs can precisely target and inhibit an enzyme essential for the influenza virus' replication. Since all strains of the virus require the same functioning enzyme, researchers believe their findings will yield drugs that can effectively treat new strains of the virus, which may be resistant to current antiviral treatments.

When new strains of influenza emerge, it can take many months for a vaccine to be developed. Experts are concerned that the emergence of any highly virulent strains could result in large numbers of people being hospitalized, and if the strain is or becomes resistant to current treatments, the impact could be catastrophic. The researchers' findings may lead to the ability to develop drugs that not only treat influenza but impede the ability of the virus to develop drug resistance. The study appears online in the August 2 issue of *PLoS Pathogens*.

St. Jude scientists tested drugs aimed at blocking a dual-purpose enzyme complex, called a polymerase, produced by the influenza virus. This polymerase produces copies of the viral genome during replication. It also assembles molecules called messenger RNA (mRNA) that code for viral proteins the virus needs to hijack the cell's machinery to make it produce more virus.

The drugs that investigators tested target an RNA-snipping enzyme called an endonuclease that is a key subunit of the polymerase complex.

The endonuclease enables the virus to disguise its messenger RNA so it will be incorporated into the cell's protein-making machinery. This masking consists of snipping apart cellular mRNA, but retaining a segment called a "cap" that the cell's machinery uses to identify its mRNA. The polymerase then attaches this cap to its own mRNA.

"Inhibitors of the polymerase complex would make excellent drug candidates," said Stephen White, DPhil., chair of the St. Jude Structural Biology department and the study's senior author. "It is a good target because these polymerases are essentially the same across many strains, and also because the virus absolutely needs the polymerase to make copies of itself. The polymerase doesn't have very many similarities to other polymerases in cells, so it should be fairly specific for the flu polymerase."

By contrast, White said, viruses have readily developed resistance to antiviral drugs currently on the market, because they target viral proteins that a virus can readily alter without compromising its viability.

In the study, the scientists drew on previous research that had mapped the molecular structure of the endonuclease, which revealed its active site. This is the region in which the chemical reaction of snipping apart mRNA molecules occurs. To test the compounds, the St. Jude researchers engineered a version of the endonuclease designed to enable them to readily determine whether compounds would block the active site.

They tested inhibition of the active site by six compounds known or predicted to block the active site. Three of the compounds had been developed earlier by Merck as viral inhibitors, and although they had some effectiveness, their properties ruled them out as drug candidates. Three other compounds predicted to block the site were synthesized in the laboratory of study co-author Thomas Webb, Ph.D., a member of the

Chemical Biology and Therapeutics department at St. Jude. As a basis for the compounds, Webb constructed a molecule dubbed a warhead, tailored to fit precisely into a central pocket of the active site. This warhead will provide the basis for generating antiviral drug candidates, which will have additional molecular segments designed to fit into neighboring pockets.

After confirming that the six compounds indeed showed activity against the endonuclease, the researchers next performed a detailed structural analysis of how the compounds fit the active site. They used X-ray crystallography, a widely employed analytical technique in which X-rays are directed through a crystallized protein, and the pattern of diffracted X-rays analyzed to deduce the structure.

Those studies confirmed that the warhead binds to the active site and also yielded new information on other surrounding pockets into which the compounds fit. The study also revealed which pockets are conserved among different strains.

“By analyzing the structure of the active site with the drugs bound to it, we have identified a number of pockets inside the active site of the protein,” said study first author Rebecca DuBois, Ph.D., a postdoctoral research fellow in the St. Jude Structural Biology department. “We can use these structures to develop drugs that will specifically target certain pockets. Now that we know which pockets are really conserved, we can predict the best way to avoid the development of resistance by viral strains.” Such resistance arises when a viral strain mutates to change the structure of a pocket, eliminating the ability of a drug to bind and block the active site.

Next, the researchers will use this information to design improved compounds that could be drug candidates for pre-clinical and clinical testing, according to White. The St. Jude team will work with a

pharmaceutical company to further develop and test drugs, as part of a collaboration headed by Webb and supported by the National Institutes of Health.

Once in clinical use, the drugs would offer a valuable and widely used first strike against the virus. “You could use these drugs in any situation where a person is hospitalized for influenza,” DuBois said. “Whether used alone or in combination with existing influenza medications, we would expect them to be highly effective.”

Other viruses, such as Hantavirus and lymphocytic choriomeningitis virus, have polymerases that function like that of the influenza virus, DuBois said. Thus, the drug molecules designed for influenza could likely be adapted to treat such viruses.

The study’s other authors are Jake Slavish, Brandi Baughman, Mi-Kyung Yun, Ju Bao and Richard Webby.

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