Researchers discover new antituberculosis compounds

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Attempts to eradicate tuberculosis (TB) are stymied by the fact that the disease-causing bacteria have a sophisticated mechanism for surviving dormant in infected cells. Now, a team of scientists led by researchers from Weill Cornell Medical College has identified compounds that inhibit that mechanism -- without damaging human cells. The results, described in the next issue of *Nature* and published online today, include structural studies of how the inhibitor molecules interact with bacterial proteins, and could lead to the design of new anti-TB drugs.

"We believe these findings represent a new approach for developing antibiotics in the fight against TB," says Dr. Carl Nathan, senior author and chairman of the Department of Microbiology and Immunology, R.A. Rees Pritchett Professor of Microbiology and director of the Abby and Howard P. Milstein Program in Chemical Biology of Infectious Disease at Weill Cornell Medical College. "This is important because we are running out of effective antibiotics that are currently available. There are few drugs that successfully combat TB in its dormant stage, which makes the bacterium so resilient in the body. More important, there are many antibiotics that kill bacteria by blocking the synthesis of proteins, but there are none that kill bacteria by interfering with protein breakdown, as we have found here."

Mycobacterium tuberculosis, the bacterium that causes TB, infects one person in three worldwide. Most infected people remain symptom-free because the bacterium is kept in check within immune system cells. These cells produce compounds such as nitric oxide, which scientists

believe damage or destroy the bacteria's proteins. If allowed to accumulate, the damaged proteins would kill the bacteria.

But TB bacteria have a sophisticated way to remove the damaged proteins -- a protein-cleaving complex known as a proteasome -- identified in earlier research by the Nathan lab. By breaking down damaged proteins, the proteasome allows the bacteria to remain dormant, and possibly go on to cause active TB. Finding drugs to disable the proteasome would be a new way to fight TB.

In developing proteasome-inhibitor drugs, scientists face several hurdles. A significant one is the fact that human cells also possess proteasomes, which are essential to their survival. To be effective, the drugs would have to specifically target the TB proteasome without adversely affecting the human protein-cleanup complex.

"This study represents a shift in strategy for designing antibiotics that treat TB," says Dr. Lin, first author and assistant research professor of Microbiology and Immunology at Weill Cornell Medical College. "Before now, researchers focused on developing drugs that attacked the bacterium in its active phase, but our group has found a compound that may help to destroy it in its dormant stage."

The Weill Cornell team screened 20,000 compounds for TB proteasome inhibition activity. They identified and synthesized a group of inhibitors, which they then tested for their ability to inhibit the proteasome inside the mycobacteria. They also tested the compounds' effect on monkey epithelial cells and human immune system cells in culture.

Screening was conducted at the High Throughput Screening Resource Facility, a jointly funded collaboration between Weill Cornell Medical College and Rockefeller University. In addition, Dr. Haiteng Deng, director of the Proteomics Resource Center of Rockefeller University,

helped analyze the mechanism of inhibition.

A series of related compounds designed by Dr. Lin and synthesized in the Milstein Chemistry Core Facility at Weill Cornell proved to be effective against the TB bacteria while showing no apparent toxicity to mammalian cells. Additionally, the compounds exerted no antibacterial activity against a range of other bacteria, demonstrating that they appear to have a high degree of specificity for the TB microbes. Furthermore, the inhibition of the TB proteasome is irreversible and about 1,000-fold more effective than the minor inhibition observed against human proteasomes.

To learn more and confirm the inhibitory mechanism and the basis for its species selectivity, Dr. Huilin Li, co-author and biophysicist from Broohaven National Laboratory in Upton, New York, and his group determined the atomic-level crystal structures of TB proteasomes following exposure to the inhibitors. These studies were performed at the National Synchrotron Light Source (NSLS) -- a source of intense x-ray, ultraviolet, and infrared light beams at Brookhaven Lab.

The structural studies revealed that the inhibitor molecules block the proteasome's ability to degrade proteins in more than one way: by producing a direct chemical change to the proteasome active site, and by altering the conformation of the "pocket" into which protein fragments bind before being degraded.

"This conformational change constricts the pocket to the point that it cannot accommodate a protein substrate," said Li. "The many amino acid residues of the TB proteasome involved in this conformational change, some far away from the active site, are different from those in human proteasomes. This might explain why such dramatic inhibition is not observed in the human proteasome, as the human enzyme may not be able to undergo the same structural change."

A detailed understanding of the steps by which these inhibitors cause the conformational changes could therefore guide the design of the next generation of anti-TB drugs.

Source: New York- Presbyterian Hospital (news : web)