Mass spectrometry helps researchers 'watch' how antibiotics attack tuberculosis bacteria inside cells

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Weill Cornell Medical College researchers report that mass spectrometry, a tool currently used to detect and measure proteins and lipids, can also now allow biologists to "see" for the first time exactly how drugs work inside living cells to kill infectious microbes. As a result, scientists may be able to improve existing antibiotics and design new, smarter ones to fight deadly infections, such as tuberculosis. The new study was published in today's early online edition of *Science*.

"The development of antibiotics has been stalled for several decades and many infectious microbes have become drug-resistant," says the study's senior investigator, Dr. Kyu Y. Rhee, an infectious disease expert who is an associate professor of medicine in the Division of Infectious Diseases and associate professor of microbiology and immunology at Weill Cornell Medical College. "We must restock the antibiotic pipeline and our study findings provide a powerful new approach for doing just that."

The need to develop new antibiotics is perhaps nowhere more pressing than for the treatment of tuberculosis, TB, which is the single leading bacterial cause of death worldwide, and with the emergence of now total drug resistance, an unchecked global public health emergency.

"Current TB treatments are long and complex, lasting a minimum of six months, and often resulting in treatment failures and the paradoxical emergence of multi-drug resistance," says Dr. Rhee, who is also an

associate attending physician at NewYork-Presbyterian Hospital/Weill Cornell Medical Center. "We are still using the antibiotics that were first developed for TB about 50 years ago."

Most TB drugs—as well as antibiotics for other infections—were developed through a combination of empirical approaches, Dr. Rhee explains. "However, it had been impossible to know what the drug was doing inside the bacteria."

That situation has now changed. Dr. Rhee and his colleagues, who include investigators from the National Institutes of Health, applied modern technologies that stem from use of mass spectrometry to directly visualize what happens when these drugs infiltrate TB cells. They can "watch," at a basic biochemical level, what happens to both the antibiotic agent and infecting bacteria over time after the drug is administered.

Mass spectrometry, simply stated, is a tool that weighs individual molecules as a way to identify them. It was first used in physics, but has expanded to many disciplines to help scientists identify molecules and determine the quantity of each kind in gases, liquids, as well as solids. Advances in mass spectrometry have made it possible for biologists to leverage the tool in the last few years, and, with this study, evaluate the intracellular fates and actions of small drug molecules.

This study is the first to show mass spectrometry can also be adapted to understand the action of antibiotics on living, intact bacterial cells.

In the study, Dr. Rhee's research team exposed TB to para-aminosalicylic acid (PAS), which was developed more than 50 years ago, and is still part of the multi-drug regimen used to treat resistant TB. It is the second oldest TB drug on the market.

The drug was thought to work by inhibiting an enzyme used by bacteria

to synthesize folates, an essential class of nutrients that humans acquire by eating, but bacteria must make on their own. "Many thus believed that the drug interfered with folate synthesis in the TB bacterium by functioning as an occlusive plug that blocked this pathway," says Dr. Rhee.

However, researchers actually found, while it is true PAS prevents the utilization of the natural precursors used to synthesize folates, once inside TB, PAS itself also turns toxic. "PAS is an agent that uses the TB cell's machinery to turn it into a poison. Thus, it doesn't simply kill the cell by stopping its food supply, it also morphs into a lethal drug," Dr. Rhee says.

The researchers also tested a different drug, sulfonamide (sulfa), which is an 80-year-old class of antibacterial agents known to defeat many infections, but not TB successfully.

"Scientists thought sulfa didn't penetrate TB cells, but we witnessed, using mass spectrometry, that it did, in fact, enter the bacteria. But that once inside, TB bacteria were able to degrade the drug," Dr. Rhee says. This finding suggests to researchers that it might be possible to modify the sulfa molecule so that it can withstand degradation by TB bacteria.

"Both of these findings were completely unexpected," says Dr. Rhee. "The study findings show us that sometimes there is a profound disconnect between what we think a drug is doing and how it actually works inside cells."

"The power of mass spectrometry is now evident, and we can't wait to use it to test all of the current cocktail of drugs used to treat TB to find ways to improve them," Dr. Rhee says. "Best of all will be the use of this tool to design and test the much-needed next generation of effective anti-TB agents."

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