New class of antibiotics may lead to therapy for drug-resistant tuberculosis

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A team of Rutgers University scientists led by Richard H. Ebright and Eddy Arnold has identified a new antibiotic target and a new antibiotic mechanism that may enable the development of broad-spectrum antibacterial agents effective against bacterial pathogens resistant to current antibiotics. In particular, the results could lead the way to new treatments for tuberculosis (TB) that involve shorter courses of therapy and are effective against drug-resistant TB.

The researchers showed how three antibiotics – myxopyronin, corallopyronin and ripostatin – block the action of bacterial RNA polymerase (RNAP). RNAP is the enzyme that transcribes genetic information from DNA into RNA, which, in turn, directs the assembly of proteins, the building blocks of all biological systems. Blocking bacterial RNAP kills bacterial cells.

The research findings are reported in the journal *Cell*, published online Oct. 16 and in the Oct. 17 print issue of the journal.

The shape of the RNAP molecule is key to the action of the three antibiotics, Ebright explained. "RNAP has a shape reminiscent of a crab claw, with two prominent pincer-like projections," he said. "Just as with a real crab claw, one pincer stays fixed and one pincer moves – opening to allow DNA into the enzyme and closing to keep DNA in the enzyme. The pincer that moves does so by rotating about a hinge, termed the 'switch region,' located at its base."

The studies showed that the three antibiotics bind to this hinge and, further, that by jamming the hinge, they prevent the pincer from opening to let DNA into the enzyme, Ebright said.

Once the target and mechanism of the three antibiotics were elucidated, the researchers proceeded to determine the structure of RNAP bound to one of the three antibiotics. "This has allowed us to define how the enzyme and the antibiotic interact and to characterize how the enzyme changes shape in response to the antibiotic," Arnold said. "Perhaps more important, this has allowed us to explore ways to change the chemical structure of the antibiotic to make tighter interactions with the enzyme for higher potency."

The three antibiotics exhibit potent activity against a broad spectrum of bacterial species, including the bacterium that causes TB, and exhibit no cross resistance with current antibacterial agents.

"The three antibiotics are attractive candidates for development as broad spectrum antibacterial agents," Ebright said, "and their target within RNAP – the hinge or 'switch region' – is an attractive target for identification of new broad-spectrum antibacterial therapeutic agents."

Arnold points out that the binding site for the three antibiotics has attractive features for design of new agents. "The target site is a pocket that accommodates a variety of chemical types. The nature of the binding site and mechanism of inhibition are analogous to those of the HIV-1 reverse transcriptase non-nucleoside inhibitors, which include four FDA-approved drugs for treating HIV-1 infections. The parallels are encouraging and suggest that multiple classes of agents can be developed to target the new site."

Ebright, a Howard Hughes Medical Institute investigator, is a professor in the Department of Chemistry and Chemical Biology and a member of

the Waksman Institute of Microbiology at Rutgers, The State University of New Jersey. Arnold, also a professor of chemistry and chemical biology, is a member of the Center for Advanced Biotechnology and Medicine (CABM), jointly operated by Rutgers and the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School.

Jayanta Mukhopadhyay from Ebright's laboratory and Kalyan Das from Arnold's laboratory carried out much of the work.

The research team also included Rolf Jansen and Herbert Irschik of the Helmholtz Center for Infection Research in Braunschweig, Germany.

Most antibacterial compounds are able to kill actively growing TB bacteria but are unable to kill resting, dormant TB bacteria. As a result, most antibacterial compounds can rapidly reduce populations of TB bacteria in infected patients to low numbers but cannot rapidly reduce these numbers to zero. Antibacterial compounds that target RNAP, however, are able to kill both active and dormant TB bacteria since RNAP plays essential roles in, and is required for survival of, both active and dormant TB bacteria.

A class of antibacterial compounds known as rifamycins, which target RNAP, are current first-line treatment of TB and are the sole current treatments that can relatively rapidly reduce populations of TB bacteria to zero. Unfortunately, rifamycins are too toxic to administer at doses that most rapidly clear infection. Also, resistance to rifamycins occurs frequently, due to mutations that alter their binding site on RNAP.

The three antibiotics studied by Ebright and co-workers also target RNAP; however, they target a new site on RNAP, different from the site on RNAP targeted by rifamycins. "A key point about these antibiotics is that their binding site on RNAP is different from, and does not overlap

with, the binding site for rifamycins," Ebright said. "As a result, these antibiotics can function simultaneously with rifamycins and can be co-administered with rifamycins for more rapid clearance of infection. As a further result, these antibiotics do not exhibit cross-resistance with rifamycins. Mutations that alter the binding site for rifamycins on RNAP and confer resistance to rifamycins do not confer resistance to these antibiotics.

The standard course of therapy for most bacterial infections is about two weeks, but TB is different. The shortest course of therapy for TB is six to nine months. "That is, if you can use rifamycins," Ebright notes. "If you have a patient who cannot tolerate rifamycins, or if you have a patient whose infection is resistant to rifamycins, that patient is looking at 18 to 24 months of therapy."

"The Holy Grail in TB therapy is to reduce the course of therapy from six months to two weeks – to make treatment of TB like treatment of other bacterial infections," Ebright said. "If you could develop a two-week therapy for TB, you could eradicate TB. With a six-month course of therapy for a disease that is largely centered in the third world, the logistical problems of administering therapy over space and time make eradication a nonstarter. But if there were a two-week course of therapy, the logistics would be manageable, and the disease would be eradicated."

The hope is that the new findings will bring that goal closer.

Source: Rutgers University