Probing bacterial resistance to a class of natural antibiotics

December 16 2014, by Richard Harth

Antimicrobial peptides are a distinctive class of potent, broad-spectrum antibiotics produced by the body's innate immune system—the first line of defense against disease-causing microbes.

In a new study, Yixin Shi, Ph.D., and Wei Kong, Ph.D., researchers in the Center for Infectious Diseases and Vaccinology at Arizona State University's Biodesign Institute, explore the clever techniques used by bacteria to survive destruction from antimicrobial peptides—potent defense factors produced by all living forms, including humans.

Professor Shi underscores the importance of antimicrobial peptides in the pitched battle against multi-drug resistant bacteria:

"All bacteria treated with conventional antibiotics will develop antibiotic resistance," he says. "But antimicrobial peptides have a unique function. Many of them target the bacterial membrane, making it very difficult for bacteria to develop resistance." After fusing with the invasive bacteria's membrane, antimicrobial peptides cause membrane leakage, leading to cell destruction or lysis.

The researchers describe one strategy bacteria have evolved to try to shield themselves from the effects of antimicrobial peptides, allowing the pathogens to survive efforts to eradicate them. A two-component system, used by pathogenic invaders like *E. coli* and *Salmonella*, facilitates expression of multi-drug pumps that can remove antimicrobial peptides from the bacterium's cytoplasm.

The study's findings suggest that if this two-component system could be disabled, disease-causing bacteria would fall victim to the lethal effects of antimicrobial peptides. The research helps open the door to the clinical application of these powerful antibiotics at a time when such novel therapeutics are desperately needed to stem the tide of bacterial resistance.

Scientific collaborators from ASU's School of Life Sciences as well as researchers from the College of Life Sciences, Inner Mongolia University, China and the State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, China join Drs. Shi and Kong.

The group's research results recently appeared in the *Journal of Biological Chemistry*.

A tool in Nature's arsenal

The bactericidal properties of antimicrobial peptides were first discovered when researchers sought to determine how frogs could live healthy lives in bacteria-rich ponds, seemingly immune to infection. As it happens, a frog's skin is covered with antimicrobial peptides, which lyse the bacteria they come in contact with, thereby protecting these animals.

Antimicrobial peptides have lately become the focus of intense investigations aimed at unlocking their intriguing properties. It is hoped that in the future, naturally occurring antimicrobial peptides may be used as templates for a whole new range of custom-designed therapeutics against pathogens currently resistant to mainline antibiotics.

Perhaps the most significant barriers to such an approach are the clever strategies used by bacterial pathogens to outmaneuver antimicrobial peptides and fight another day. While professor Shi studies the ways

bacteria fortify themselves against antimicrobial peptides, professor Kong explores how the bacterial resistance system may be weakened, allowing the antimicrobial peptide to function better.

Antimicrobial peptides target bacterial cells by exploiting a characteristic of their membrane physiology. Bacterial cells are prokaryotic—lacking a cell nucleus. They differ from nucleated or eukaryotic cells, (like those found in humans), in another critical respect: the membranes of bacterial cells carry a negative electrical charge compared with eukaryotic cells, which are positively charged.

This fact allows the positively charged antimicrobial peptides produced by host cells to bind with negatively charged bacterial membranes. (These positively charged natural antibiotics are often known as CAMPs, for cationic antimicrobial peptides.) As professor Kong explains, "the antimicrobial peptide then acts like a needle to pierce the bacterial cell." Various CAMPs are capable of targeting not only bacteria, but parasites, viruses, fungi and other invasive life forms.

The bugs strike back

Bacterial cells, however, have a few tricks of their own. Using the two-component system described in the new study, they are able to remove antimicrobial peptides, blocking their bactericidal effect. This two-component system—labeled CpxR/CpxA—is thought to be a very ancient adaptation, possibly used to thwart early antimicrobial peptides, which are believed to have arisen since these pathogens first developed an ability to invade their hosts.

Intriguingly, the authors suggest that the same mechanism may be used by pathogenic bacteria to pump out a broad range of human-designed antibiotics as well. The two-component system also performs a variety of important housekeeping functions, for example, helping bacterial cells

maintain the structure of their envelope and insulating them from heat shock.

In the current study, a genome-wide susceptibility assay was used to pinpoint specific genes that facilitate *E. coli* resistance to a specific CAMP known as protamine. To do this, the researchers made use of the Keio Collection, a vast bacterial library containing over 4000 individual mutants of *E. coli*. The team isolated 115 bacterial strains bearing a single deletion at a site known or predicted to affect susceptibility to protamine.

One bacterial candidate bearing deletion of a gene encoding an outer membrane component showed high susceptibility to protamine, compared with the wild-type *E. coli*. This gene, known as tolC, appears to be a vital constituent, helping *E. coli* remove protamine. The authors believe this novel mechanism initiated by a multidrug resistance cascade likely plays a role in bacterial resistance to other CAMPs.

When bacterial cells in the study were challenged with 1.0 mg/ml of protamine, roughly 40 percent of the wild-type strain survived. By contrast, the mutants bearing specific gene deletions showed heightened susceptibility to protamine, with the tolC mutant being killed completely (0 percent survival) by the same dose of protamine. Further, only 4.4 percent of the tolC mutants survived low dose (.5 mg/ml) protamine challenge.

New drugs for resisting resistance

Despite promising research, there is much more work to be done before the power of these unique antibiotics can be harnessed for clinical use. While CAMPs tend to target the negative surface charge of bacterial cell membranes, they sometimes prove cytotoxic to various host cells as well.

Antimicrobial peptides are sensitive to host proteases and also remain relatively expensive. If the bacterial resistance system can be overridden however, the bactericidal properties of CAMPs will improve and lower doses would be required for effectiveness, limiting the threat to healthy cells while reducing cost. Improving CAMP specificity for bacterial membranes will also enhance their effectiveness.

The results of the current study offer important insights into mechanisms of bacterial resistance to antimicrobial peptides like protamine while supplying tantalizing clues as to how the system might be disrupted. In the future, antimicrobial peptides may be developed into single-dose therapeutic agents capable of targeting multiple pathogenic forms including bacteria, fungi, viruses, etc. Further, combining CAMPs with existing antibiotics offers the possibility of designing a weapon with synergistic effects against infectious invaders.

Bacterial resistance continues its perilous ascent and is now considered a major threat to public health at a time when development of new drugs to address aggressive pathogens has slowed to a crawl. Antimicrobial peptides open a new vista in the continuing war against disease-causing bacteria and other threats to human health.

Provided by Arizona State University