Unique Microbe Killer Could Transform Animal And Human Health

Brigham Young University

Thanks to an unlikely pairing of horse breeders and chemists at Brigham Young University (BYU), a new antimicrobial therapy is delivering dramatic results to animals plagued by persistent bacterial infections. The technology, which is also being tested for human pharmaceutical and medical applications, may be an answer to the growing problem of antimicrobial-resistant (AMR) infections.

Too Much of a Good Thing

The introduction of antimicrobial drugs in the early 20th century was a major milestone in the practice of modern medicine. Penicillin and other antibiotics conquered countless bacterial enemies, including pneumonia and tuberculosis, dramatically reducing the death toll from infectious diseases.

But the overuse or misuse of antimicrobials has enabled bacteria, viruses, fungi and parasites to adapt and become antimicrobial-resistant. As a result, diseases and infections once eradicated with antibiotics are becoming difficult or impossible to treat, posing a worldwide threat to both animal and human health. The World Health Organization (WHO) has warned that the 21st century may bring a post-antibiotic era in which common infections and minor injuries can kill.

Scientists, including BYU's Paul Savage, have been feverishly studying the mechanisms of bacterial growth and resistance, looking for new ways to combat AMR. After taking graduate-level classes in microbiology, Savage became interested in the immune system's first line of defense — antimicrobial peptides or AMPs.

"The National Institutes of Health offered biotech training when I was a Ph.D. student in organic chemistry," says Savage, a professor of chemistry and biochemistry. "I was exposed to bacterial processes that I began to understand at a chemical level."

Searching for Ways to Combat AMR

AMPs consist of a short chain of amino acids capable of destroying the structure of the membranes of invading microbes, leading to cell death.

"AMPs have been present for hundreds of millions of years and are everywhere in the natural world," says Savage. "AMPs have been found in practically everything studied, including humans, animals, fish, insects and plants."

In humans, AMPs are deployed at the earliest stages of infection and play a key role in innate immunity.

"AMPs are essential for human health," explains Savage. "A deficiency in AMPs on the skin is associated with atopic dermatitis infections."

> *And that's just the tip of the iceberg, according to Savage. In the absence of AMPs, periodontal disease and blood stream and urinary tract infections can run rampant.*

Savage knew that the clinical use of naturally occurring AMPs to treat bacterial infections, called peptide therapy, was hampered by several problems, including high production costs and instability. AMPs also have their own enemies — including enzymes produced by bacteria and the human body that are able to bind to and destroy peptides.

"Bacteria can release enzymes that breakdown AMPs, and we have proteases in the mouth that chew up AMPs," says Savage. "It's chemical warfare."

Mimicking Naturally Occurring Peptides

Savage and his team of graduate and postdoctoral students set out to develop a nonpeptide mimic of AMPs in the hope of avoiding the problems associated with peptide therapeutics.

He theorized that synthetic mimics of AMPs would be far less costly to prepare on a large scale and more easily controlled than natural AMPs. He also suspected that a nonpeptide mimic of the AMP would not be susceptible to the proteases that destroy AMPs.

Using a common bile acid produced by the gallbladder as a scaffolding, the researchers added amino acids and other chemical groups to create a new chemical compound.

"We looked at the shapes of AMPs and used the tools of synthetic and organic chemistry to identify what small molecules reproduced that shape," explains Savage.

A New Antimicrobial

To understand the structural features required for antibacterial activity, the team prepared hundreds of such compounds over years, ultimately identifying a new class of antimicrobial agent called the cationic selective antimicrobial (CSA) or ceragenin that was highly active against invading microbes.

The researchers found that like AMPs, the positively charged CSAs were attracted to the negatively charged cell membranes of viruses, fungi and bacteria.

“With the CSAs, we see the same mechanisms of action as with the AMPs,” says Savage. “They selectively target bacterial membranes, depolarize and kill.”

Even better, the researchers found that the CSAs had distinct advantages over natural AMPs: As expected, they were far more scalable and less costly to produce. Further research revealed the synthetic compounds were also able to prevent the formation of biofilms — otherwise known as that slimy stuff that grows on the side of the water bowl.

Fighting Communities of Bacteria

“Bacteria grow in their own communities, creating biofilms that are not very susceptible to antibiotics,” says Savage. “Our arsenal of antibiotics that works on biofilms is near zero.”

Biofilms can grow on catheters and tubing used to deliver oxygen and fluids to patients — and even on medical devices implanted in patients, such as artificial hips.

“Persistent infections, such as *Pseudomonas aeruginosa* (a common bacteriumin the lungs of cystic fibrosis patients), contain biofilms and are almost impossible to eradicate,” he says.

With the help of BYU’s Technology Transfer Office, the family of CSA compounds (which numbers in the hundreds) was patented in 1997. Today, there are a total of 35 patents related to the technology in the United States and 16 countries abroad.

“Dr. Savage is top-notch, he could be at any research university in the U.S., but he’s chosen to be at BYU,” says G. Michael Alder, director of technology transfer at BYU.

“He and other chemistry faculty love the working environment [at BYU] and are bringing us great new inventions.”

A Broad-Spectrum Antibacterial

With help from other academic and commercial laboratories, Savage determined that the family of CSA compounds displays a broad spectrum of antibacterial activity, including against gram-positive, gram-negative and drug-resistant bacteria. Some 48 peer-reviewed articles have been published confirming the efficacy of the antimicrobial, which can be formulated and added to topical creams and gels, encapsulated and swallowed, or used as a coating.

“I’m excited that we can move forward with confidence, knowing we have the data that shows good efficacy, no toxicity, and strong upsides with few downsides,” says Savage.

After the first company to license the technology in 2006 went bankrupt, BYU found a second licensee in 2010 — a company called N8 Medical — for the rights to human and pharmaceutical applications. A second company, Diamond Fork, licensed the family of CSA compounds for use in the animal industry, which is how horse breeders Lance Robinson and Chad Beus were introduced to the technology.

In 2011, Robinson and Beus had a bacterial infection in their barn when they were approached by Diamond Fork to try a spray containing the CSA compound. They were losing foals — a costly problem for the breeders, who command as much as $1 million for a full-grown thoroughbred or quarter horse.

“We did a trial, spraying the barn, mares and foals every other week and all of our foals got well,” says Robinson.

From Horse Breeding to Biopharmaceuticals

The breeders were so impressed with the results they formed a company called CSA BioTech two months later and bought into the Diamond Fork business. But they quickly decided to buy out the company, gaining all animal and disinfectant rights to the CSAs.

“Because of the success Chad and I have had in the horse industry, we’ve always had people asking us to test products and this is the only thing we’ve ever used that worked like this,” says Robinson. “We knew this was something special, a game changer.”

In 2013, N8 Medical sold the majority of its rights for pharmaceutical and human applications to CSA Biotech, retaining a few shared pharmaceutical applications with CSA and applications for medical devices.Today, N8 is focusing on its work on developing both its medical device coating, as well as treating cystic fibrosis with CSA therapy.

CSA BioTech

With the help of a group of investors, Robinson, Beus and a third managing director, Mike Moore, are working on taking CSA Biotech in several directions. Through the subsidiary company BioCare Animal Products, they are selling topical, wash and spray treatments for cats, dogs and horses. The retail products, sold under the brand name Purishield, are available both online and at retail outlets throughout the country. A second subsidiary, Ceragyn, sells veterinary-grade formulations of the antimicrobial.

“Our Purishield wound spray has done well,” says Robinson.“[At Ceragyn], our No. 1 product is a uterine lavage (in which the uterine cavity is irrigated with the therapeutic solution) to address reproductive problems in equines, treating mares that have chronic infections in the uterus.”

Meanwhile, the company is making progress on human preclinical studies of the antimicrobial to treat chronic wounds, such as diabetic foot and venous leg ulcers and herpes. CSA BioTech is also capitalizing on the selectivity of the ceragenins by testing their ability to destroy several types of cancer, including breast (through an intravenous injection), skin (a topical treatment) and bladder (delivered as a lavage).

“The ceragenin technology is selective, it does not attack healthy cells,” says Robinson.

With just eight employees, the Utah-based CSA BioTech relies heavily on contracted labor, which has been key to the company’s success, according to Robinson. The company, which works with researchers in the United States and abroad, has a close relationship with the School of Veterinary Medicine at the University of Wisconsin-Madison.

Robinson said human applications are mostly likely to become available first in Latin America and the European Union, followed by the United States.

“I never would have guessed this is where I’d end up,” says Robinson, who now devotes 90 percent of his time to the new businesses. “Dr. Savage has created something unique. This was completely outside our area of expertise, but we knew this was something special, [worth a] complete change of business and life.”

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