Beyond Vancomycin

Understanding an antibiotic of last resort may lead to new weapons against deadly bacteria

By CORINNA WU

For years, doctors counted on the antibiotic vancomycin to wipe out infections caused by *Staphylococcus aureus*, a dangerous bacterium that had developed resistance to every other drug. Their confidence was shattered 3 years ago when a month-long treatment with vancomycin failed to cure a 4-month-old boy in Japan who had acquired an *S. aureus* infection after heart surgery. Only a combination of drugs finally subdued the infection (SN: 6/7/97, p. 348).

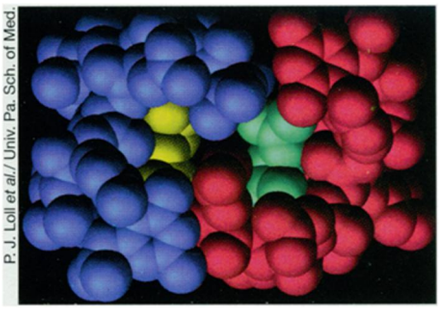
More recently, stubborn forms of *S. aureus* popped up close to home. Researchers in the United States described three cases of vancomycin-resistant *S. aureus* in the Feb. 18 NEW ENGLAND JOURNAL OF MEDICINE (NEJM). The patients—from New York, Michigan, and New Jersey—were being treated in the hospital for various ailments, including kidney failure. In two of the patients, the *S. aureus* infection was eradicated, but all three died nevertheless (SN: 3/13/99, p. 175).

The discovery of these strains has sent chills up the spines of public health officials. For a long time, vancomycin has held back the floodwaters of epidemics caused by microbes. Infections with *S. aureus* and enterococcus, a group of gut bacteria, often occur in hospital patients after surgery but had always yielded to treatment with vancomycin. However, vancomycin-resistant strains of enterococcus that appeared in 1983 and now resistant *S. aureus* signal that bacteria have poked holes in the dam.

Without new weapons to kill these resistant strains, the prognosis for public health is dire. "If they don't come up with new drugs, people won't have elective surgery because it will be too dangerous," predicts Daniel Kahne, a chemist at Princeton University.

Understanding how vancomycin works and how bacteria defeat the drug has led to novel ways to improve its power. With their new knowledge, researchers hope to find even more strategies to stay one step ahead of these lethal bugs.

Ironically, killing bacteria with drugs is what creates the problem of resistance in the first place. In 1942, the very same year that the most commonly used form of penicillin was developed, strains of *S. aureus* were found to be resistant to it.



P. J. Loll *et al.*/ Univ. Pa. Sch. of Med.

Computer models of vancomycin's three-dimensional structure help scientists examine how the drug binds to components of a bacterial cell wall.

"Bacteria have an innate ability to develop resistance," says Marissa Miller, a program officer at the National Institute of Allergy and Infectious Diseases in Bethesdà, Md. Any population of bacteria naturally harbors a range of genetic variations that influence resistance to antibiotics. Microorganisms susceptible to an antibiotic succumb, but the hardier ones survive and multiply, passing on their resistance to subsequent generations.

Excessive use of antibiotics has worsened the problem. The Centers for Disease Control and Prevention in Atlanta estimates that half of the 100 million courses of antibiotics prescribed by doctors each year are unnecessary. These drugs are useless against the common cold and other viral infections, but patients often pressure doctors to prescribe them. For example, a survey published in the February PEDIATRICS found that 96 percent of pediatricians polled had been asked by parents to prescribe antibiotics during the previous month.

The livestock industry, too, contributes to the trouble. It often gives antibiotics to animals in low doses to promote growth. These doses, not high enough to kill all the bacteria present, enhance the development of resistance, says Miller.

Although vancomycin is not carelessly prescribed for patients or used in livestock, the increasing resistance to more common antibiotics requires doctors to turn to their antibiotic of last resort more and more often.

Limiting unnecessary antibiotic use can help stem the tide, but doctors still need more tools to fight the resistant strains that will inevitably crop up. In the 1980s and early 1990s, however, research into new antibiotics lay dormant.

"Drug companies were not that interested because we thought we had plenty of great antibiotics," Kahne explains.

The incidence of vancomycin-resistant enterococci has jumped from less than 1 percent to about 15 percent in the past dozen years, says Christopher T. Walsh of the Harvard Medical School in Boston. "There's the worry that genes will hop from enterococcus to *Staph. aureus*, which is much more virulent." Such a transfer has occurred in test-tube experiments.

So far, *S. aureus* isolated from patients has only shown an intermediate-level resistance to vancomycin, meaning that most infections can still be halted with a moderately increased dose of the drug.

Vancomycin works by interfering with the way bacteria build their cell walls. By grabbing onto the ends of cell wall components known as peptidoglycans, the antibiotic prevents them from linking together and thus weakens the wall's structure.

In resistant strains of enterococci, however, the ends of the peptidoglycans have changed, reducing vancomycin's ability to bind. Instead of terminating with a unit made of two copies of the amino acid alanine, the resistant peptidoglycans end with an alanine and a molecular group called lactate. These modified molecules are only one-thousandth as sensitive to vancomycin as the more common ones, Walsh says.

He and his colleagues found that a group of five genes is necessary for these changes. Two genes make proteins that detect vancomycin and turn on the other three genes. Those three genes code for enzymes that "reprogram the cell wall" by providing the new peptidoglycan end segment, Walsh says. "If you find ways to block any of the three enzymes, then you could reverse the behavior." Researchers are now trying to find molecules that can do just that.

Even though vancomycin has been available for 40 years, researchers only recently found ways to make it from scratch. Pharmaceutical companies have been making the compound commercially by isolating it from a fungus that produces the drug naturally.

Vancomycin is a cup-shaped molecule consisting of two sugars attached to a larger complex of amino acids known as the aglycon. In October 1998, a team led by K.C. Nicolaou of the Scripps Research Institute in La Jolla, Calif., and another led by David A. Evans of Harvard University reported that they had succeeded in assembling the aglycon, which is made up of seven amino acids joined together in linked rings.

The Scripps team then successfully attached the sugars, completing the synthesis. The researchers reported their results in the Jan. 15 ANGEWANDTE CHEMIE INTERNATIONAL EDITION.

Understanding how to assemble the structure "is a prelude to gaining the ability to construct our own molecules," says Nicolaou. By changing key parts of vancomycin, researchers might be able to create more effective versions of the drug.

Most of this work focuses on replacing the sugars, since they appear to be important to the drug's activity. Scientists at Eli Lilly have already modified the sugars on mold-produced vancomycin and are testing a variant in people. So far, their new antibiotic has shown greatly enhanced activity against vancomycin-resistant enterococci infections.

Still, "it wasn't obvious why these carbohydrate derivatives of vancomycin would have such good activity, especially given the mechanism that Walsh worked out," says Kahne. Now, he and his colleagues, working with a group from Merck Research Laboratories in Rahway, N.J., have found a possible answer.

When the researchers damaged the aglycon portion of the vancomycin derivative, rendering it unable to bind, the altered molecule still killed bacteria effectively. That result focused their attention on the role played by the two sugars, known as the disaccharide. Kahne and his coworkers synthesized the disaccharide and tested it alone against resistant bacteria. They found that it worked 10 times better than ordinary vancomycin.

It now appears that vancomycin kills bacteria two different ways. While the aglycon portion binds to peptidoglycans, the sugars interfere with cell wall synthesis via a second pathway. In the April 16 SCIENCE, the researchers suggest that the sugars inhibit enzymes called transglycosylases, which help connect the cell wall components together.

These results "suggest new targets to kill resistant organisms that may not have been considered before," Kahne says.

Although changing the sugars on vancomycin is a grueling, 10-step process, it's still easier than trying to modify the aglycon to make it bind to a new molecule, Kahne says. Combinatorial chemical methods that substitute many different variations of the sugars onto the aglycon might yield promising candidate antibiotics. Nicolaou, Kahne, and others are avidly pursuing this approach.

The resistance mechanism of *S. aureus* remains unknown since only a few examples have turned up. The discovery of these persistent strains troubles doctors, though, because 95 percent of *S. aureus* strains already resist beta-lactam methicillin. This variation of penicillin was widely used after the bacteria developed resistance to several other antibiotics in the early 1960s.

In one of the cases described in NEJM, Alexander Tomasz of the Rockefeller University in New York and his colleagues observed that *S. aureus* developed intermediate-level vancomycin resistance in their patient while he was being treated with the drug. They had administered it for 6 weeks, taking samples of the bacteria at the beginning of the therapy and 5 weeks after it stopped. Only the final *S. aureus* sample showed resistance to vancomycin.

Disturbingly, the cells taken from the earlier samples easily developed resistance when grown in the test tube. With time, larger doses of vancomycin were required to kill them.

Tomasz and his group, however, did report some good news. A combination of vancomycin and a second antibiotic, oxacillin, killed even the most resistant *S. aureus* in their laboratory tests. If that therapy could work in a patient, it would be a reasonable way to treat these infections, the researchers say.

"Has the nightmare arrived? Not yet," says Walsh. However, the clinical cases recently observed and the danger of genes hopping from enterococci to *S. aureus* fuel an urgent need to beef up the arsenal of antibiotics, he says.

The only way to circumvent this cruel cycle, Miller says, is to develop vaccines or novel therapies (see sidebar) that treat infections without invoking the resistance associated with antibiotics.

For each new antibiotic that researchers have invented with the investment of much time, money, and effort, bacteria have quickly developed means to survive it. Dealing with antibiotic resistance recalls the punishment of Sisyphus, who was condemned to push a boulder up a hill only to have it roll down again. □

Weakening a bacterium's punch

If the bacterium *Staphylococcus aureus* had a motto, it would probably be: "What doesn't kill me only makes me stronger." Thus, instead of trying to destroy *S. aureus* outright, perhaps blocking the mechanisms that make it so deadly could better combat infections. Richard P. Novick of the New York University Medical Center, Tom W. Muir of the Rockefeller University in New York, and their colleagues are exploring this strategy.

S. aureus releases toxins that attack white blood cells and degrade tissues, producing pus-filled abscesses on the skin or in internal organs. The infection can worsen to toxic shock syndrome. Several years ago, Novick's group found a set of genes that regulates the system responsible for triggering the toxin release.

When the scientists introduced mutations into these crucial genes, they created bacteria only one-hundredth as virulent as the original ones, says Novick. "They still cause infection, but you need more."

Investigating further, the researchers focused on a peptide, produced by two genes in the set, that binds to a receptor on the bacterium's surface. Various strains of *S. aureus* produce different versions of this peptide, with lengths ranging from seven to nine amino acids. However, they all share one characteristic: Each has a cysteine amino acid in the same spot near its end.

The sulfur atom in cysteine often links parts of molecules, so the researchers hypothesized that the cysteine joins to the end of the peptide, forming a ring with a tail. It was a daring suggestion because "such a structure has never been seen in a bioactive molecule," Muir says.

By synthesizing molecules similar to the peptide and testing how well they bind to the receptor, Muir and Novick's group confirmed their novel idea. Linear molecules didn't activate the receptor. Ring-shaped molecules with an oxygen or nitrogen replacing the sulfur didn't work, either. The team reported its findings in the Feb. 16 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

Only the peptide specific to a particular strain of *S. aureus* will switch on its receptor, but the peptides from other strains can switch it off. "If you can harness this inhibition," Muir suggests, "you have a strategy to inhibit virulence."

In preliminary tests, the researchers injected a synthetic peptide corresponding to one strain of *S. aureus* into mice infected with a different strain. The peptide dramatically reduced abscesses on the backs of the mice—shrinking them almost to the small size observed when virulence genes in *S. aureus* have been completely deleted.

Other scientists have proposed that blocking the virulence mechanism could avoid the resistance problem, but Novick "isn't sure about that." Nevertheless, he thinks that such a strategy could enhance an antibiotic's effectiveness.

The bacteria produce only tiny amounts of these potent peptides, so it's almost impossible to isolate them in quantities large enough to study, says Muir. Without the chemical synthesis methods developed by Muir, Novick says, "we're nowhere." —*C.W.*