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Innovations

Holding Back the Tide of Antibiotic Resistance

Until the wide production of penicillin in the 1940s, bacterial infections were a prominent cause of deaths and disabilities around the world. Hospitals focused mainly on treating infectious diseases such as pneumonia, syphilis, tuberculosis, and other common infections. Penicillin, effective against a broad spectrum of pathogenic bacteria and with low toxicity to humans, literally revolutionized the way infectious diseases were treated and set the model for all of modern chemotherapy. As use of penicillin and penicillin-based antibiotics spread, deaths from bacterial diseases were reduced to such an extent that most infections are now considered inconvenient, rather than life threatening.

But as antibiotic use became routine, an alarming trend emerged. Bacteria rapidly developed resistance to penicillin, and cases that used to require a single shot of penicillin to cure were requiring longer treatment periods and newer drugs. In recent years, several strains of bacteria emerged that are resistant to most common antibiotics. Many physicians and scientists foresee a major crisis.

The Centers for Disease Control and Prevention estimates that every year, 2 million Americans acquire infections while in hospitals and 90,000 die and that about 70% of the hospital infections are resistant to at least one class of antibiotics [1]. Most of the multidrug-resistant infections have been postsurgical and posed a threat to the immunocompromised and the seriously ill. However, now we are seeing an increase in drug-resistant community-acquired infections, picked up not just in hospitals and nursing homes but in the places where we live and work.

Dr. Stuart Levy, professor of Molecular Biology, Microbiology, and Medicine at Tufts University and cofounder of Paratek Pharmaceuticals, a Boston biotech company that develops antibiotics to combat drug-resistant bacteria, claims that antibiotic resistance is not just a problem for the immunocompromised. He cites the examples of *Streptococcus pneumoniae*, which can cause ear infections in normally healthy children, and *Staphylococcus aureus*, a common skin pathogen. Both can lead to flesh-eating bacterial infections and are multidrug resistant. They have the potential of infecting healthy people—and they are spreading.

"With antibiotics, it is like we are always chasing our tail. We find one, we overuse it, we create resistance. We are back at square one," Dr. Stuart Levy said.

No New Drugs

Since the advent of penicillin, a dozen chemically different antibacterial structural scaffolds have been discovered. These scaffolds function as building blocks for numerous optimized antibiotics and antibacterial drugs. Despite the emerging need and intensive research, only two truly novel chemotype scaffolds, the oxazolidinone core linezolid (Zyvox) and the lipopeptides (Cubicin), have emerged in the last thirty years; and Zyvox, Cubicin, and Ketek (an antibacterial drug of the ketolide family) are the only novel medicines that have been approved by the FDA and reached the market in that time [2]. There have been many different types of antibiotics or antimicrobials, which are synthetic antibiotics, approved for the market. Most have been variants of existing drugs or cocktails of drugs with complementary or synergistic effects that were optimized to work better for certain infections. In these cases, the mechanism of action or group of bacteria targeted is not novel.

According to Levy, the discovery and synthesis of antibiotic/antibacterial drugs and the process of bringing them to market is extremely costly. Over the years, the major breakthroughs and discoveries were made by large pharmaceutical companies. Now half of the large pharmaceutical companies have left the antibiotics field for more lucrative pastures. As a result, conventional avenues for research and development of new antibiotics and antibacterial drugs have atrophied.

"The low-lying fruit have been discovered. It has been 20 years we've been looking at genes and genomes, but we haven't really come up with a new antibiotic by that approach," Levy says. Biotech companies like Paratek are filling the void. Some big pharmaceutical companies have stayed in the game by either sponsoring or working with biotech companies.

Chasing Our Tail

Antibiotics work against bacteria via various mechanisms: interfering with protein synthesis (eg., tetracycline), DNA synthesis (eg., ofloxacin), blocking cell wall synthesis (eg., amoxicillin), cell membrane permeability (eg., colistin), inhibiting an enzyme (eg., cotrimoxazole), or membrane disruption (eg., daptomycin). Correspondingly, antibiotic resistance can evolve by several paths. Bacteria can develop the ability to dispose of antibiotics efficiently. Genetic diversity in the bacteria population can cause natural variation at the antibiotics' point of action; as a result, the effectiveness of the antibiotics on the individual bacterium varies, and natural selection for the more resistant bacteria occurs. Sometimes mutation renders the antibiotics' point of action completely ineffective. The antibiotics that inhibit protein synthesis on the ribosome are some of the

hardest drugs for bacteria to build up resistance to because the chances that this very central mechanism would be totally modified by mutation are low.

Clinicians and researchers are working together to develop the next generation of antibiotics. To prevent resistance from occurring, clinicians strive to administer antibiotics as specifically targeted to the pathogen as possible (the worst strategy is to administer broad spectrum antibiotics) and give enough of a dose long enough to assure complete healing. But how do researchers develop resistance-proof antibiotics? "I think we are hearing and reading more about searching for alternatives to antibiotics," Levy says. "With antibiotics, it is like we are always chasing our tail. We find one, we overuse it, we create resistance. We are back at square one. By targeting nonessential features of the cell, but those involved in the infection process itself, we avoid selecting resistance but prevent or limit infection." New approaches are clearly essential for preserving the standards of health care down the road.

Battle Strategies

Companies are pursuing various strategies. Among them, Paratek Pharmaceuticals, Biosignal, 4SC, and BalaPharm International are investigating regulation of transcription factors and interfering with bacterial quorum sensing; Genzyme and Cengent Therapeutics are working with toxin inhibitors; and Essential Therapeutics and Daiichi are using efflux pump inhibitors. Oscient's Ramoplanin disrupts cell walls and is currently targeting the sort of *Clostridium difficile* infections that have been killing people in hospitals [3]. Using crystallographic data, Rib-X Pharmaceuticals designs antibiotics that specifically target ribosomes. Ribonovix produces bioengineered plasmids that encode mutant rRNA (the building block of ribosomes) to aid the design of drugs that can combat specific resistance-causing mutations.

Paratek (http://www.paratek.com/) is targeting the virulence of pathogens, which is their ability to infect an organ in the body and generate disease. The technological platform of the company is based on research from Dr. Stuart Levy's laboratory at Tufts University. Levy's group discovered a class of molecular switches or transcription factors, the Mar proteins, used by bacteria to resist antibiotics and cause infection. For instance, these events in *E. coli* are regulated by proteins called MarA, SoxS, and Rob.

Paratek has shown that the functions of the Mar proteins are regulated by a group of small molecules. These small molecules can be used as drugs to inhibit the ability of the proteins to function as transcription factors. When the Mar protein is inhibited, bacteria loses their virulence. Paratek has identified several small molecules that work directly on Mar proteins from *E. coli*, *Yersinia pseudotuberculosis* (a relative of the organism that causes plague), and *Pseudomonas aeruginosa* in in vitro assays. They have also achieved proof-of-concept studies by inhibiting the ability of *E. coli* [2] and recently *Y. pseudotuberculosis* to infect the kidneys and lungs of mice, respectively. The drawback of this approach is that in some cases, infection can take place via a different biochemical route, even if a specific Mar protein is inhibited. In addition, Paratek is developing modified tetracyclines.

Bug Sex Can Kill

Another novel approach is being pursued by Wisconsin-based ConjuGon (http://www.conjugon.com/). This eight-person startup is using bacterial sexual reproduction to spread lethal genes in pathogen communities. When the lethal genes are expressed, the bacterial population dies out.

"I'm a plasmid biologist," says Marcin Filutowicz, professor of bacteriology at the University of Wisconsin-Madison, and one of ConjuGon's founders. "Bacterial plasmids are extra chromosomal elements that bacteria don't need to live. They transfer DNA molecules among bacteria. They carry genes which frequently encode for antibiotic resistance. Most of the DNA that is transferred between bacteria is done through plasmid exchange." ConjuGon engineers specific plasmids with DNA that encodes toxins as well as benign bacteria that are immune to the toxins encoded by the plasmids. When immune bacteria transfer the plasmid that encodes for the toxins to the recipient bacteria, such as a pathogen like *Pseudomonas aeruginosa,* during conjugation the toxin is expressed, killing the pathogen.

"It is a Trojan-horse-like approach where we introduce a DNA molecule from donor to recipient and then express in the recipients multiple toxins or single toxins," says Filutowicz. "When we express multiple toxins, we reduce the probability for the recipient bacteria to acquire immunity."

ConjuGon is targeting genes that encode for antibiotics that block a certain RNA polymerase at several different locations in bacteria. The company has a patent on "Sigma Binding of RNA Polymerase and Uses Therof" for its method of high-throughput screening for small-molecule drugs that interfere with the binding of transcription factor sigma to the core RNA polymerase. ConjuGon chose this target because it is unlikely that bacteria would mutate in enough multiple ways to evade a compound that interferes with this interaction.

Conjugon recently received a phase I SBIR grant from the Army to do research on a treatment for antibiotic-resistant *Acineobacter baumannii* infections contracted by wounded soldiers in Iraq and Afghanistan. Additionally, Ravi Shankar, associate professor, Department of Surgery and Cell Biology, Neurobiology & Anatomy Burn and Shock Trauma Unit at Loyola University Medical Center, is conducting the proof-of-concept studies on ConjuGon's approach on mice. Shankar is interested in ConjuGon's approach as a specialized or adjunct therapy for patients with burn or trauma wounds who have a long healing process. "Much of the time, especially with burn-wound cases, it is not about life or death. It is about achieving adequate wound coverage with grafts or synthetic material so the person can heal faster," Shankar says. "But unfortunately, if you have an infection, many of the grafts fail. They lift off." Conventional antibiotic therapy can be problematic as the antibiotic has to be delivered to the actual wound area of the patient. Thus, a systemic antibiotic is not enough. A majority of time,

wound care is delivered via topical antibiotics and antimicrobials, such as topical silver emulsions.

Shankar says that in the mouse studies that pathogens were totally eliminated within 30 min of administering ConjuGon treatment. "It actually works…. We have to see if it works on human beings," Shankar says.

References

1. Bren, L. (2003). Battle of the bugs: fighting antibiotic resistance (http://www.fda.gov/fdac/features/2002/402_bugs.html).
2. Alekshun, M.N., and Levy, S.B. (2004). Targeting virulence to prevent infections: to kill or not to kill? Drug Discov. Today Ther. Strateg. *1*, 483–489.
3. Barrett, J.F. (2005). Can biotech deliver new antibiotics? Curr. Opin. Microbiol. *8*, 498–503.

Wendy Wolfson (http://wendywolfson@nasw.org) is a science and technology writer based in Oakland, CA.