INNER WORKINGS

Combating antibiotic resistance from the ground up

Elie Dolgin, *Science Writer*

Whenever anyone from Gerry Wright's laboratory goes on vacation, they come back with a small plastic bag of dirt. Usually these muddy mementos yield nothing of interest. But one graduate student's soil souvenir from a hiking trip in Eastern Canada produced a compound that could hold the key to combating one of the world's most insidious groups of superbugs.

The compound has no bacteria-killing activity itself. Rather, the agent found within the teaspoon of soil from the Kejimkujik National Park in Nova Scotia is what Wright calls an "antibiotic adjuvant." And much like an adjuvant used for boosting the immune response to a vaccine, the compound can enhance the potency of antimicrobials by, for example, blocking enzymes that serve as the linchpins for bacterial resistance.

Wright's adjuvant helped restore the killing power of the antibiotic meropenem when given to mice infected with a form of *Klebsiella* bacteria that was difficult to treat, he and his colleagues from Canada's McMaster University reported a couple years ago in *Nature* (1). The researchers have since tested the compound in rats and dogs to make sure it's safe. And a group of Boston-area entrepreneurs is now raising money to move the agent into human clinical trials. "We continue to work really hard to turn this into a drug," Wright says.

Gerry Wright and colleagues are purifying a potential antibiotic adjuvant called aspergillomarasmine A from *Aspergillus* fungus (pictured). Image courtesy of Andrew King.

With the rise of resistance to every drug in the antibiotic arsenal, and fewer than 40 new agents in clinical development, experts have warned of a coming postantibiotic crisis in which 10 million people could die globally each year from untreatable infections by 2050, unless action is taken in the very near future (2).

Finding new antibiotics is essential. But as a stopgap, it's important to "do our best to preserve the ones we have already, and the adjuvant idea is a really good one in that way," says Wright.

Rescued From Resistance

The adjuvant approach actually dates back to the mid-1970s with the discovery of the molecule clavulanic acid; several more adjuvants have followed, all of which restore sensitivity to what are known as β-lactam antibiotics. Such drugs—which include penicillins, cephalosporins, and carbapenems—contain a four-member ring called a β-lactam in their chemical structures. The adjuvants work by blocking the β-lactamase enzyme that drug-resistant bacteria use to break apart these molecular motifs.

However, these and all other β-lactamase blockers on the market suffer from a common limitation: none are effective against a particular form of the enzyme that has recently emerged as a major public health concern. This new form, known as metallo-β-lactamase, has no proven drug inhibitors. And one particularly nasty version of the enzyme—the New Delhi metallo-β-lactamase-1, or NDM-1—often spreads in tandem with other resistance mechanisms that can render bacteria impervious to nearly all available antimicrobial agents.

NDM-1 arose in India in the late 2000s and has since been found in bacteria across the world. Most of the bugs that harbor NDM-1 so far are actually not that good at causing disease, notes Timothy Walsh, a medical microbiologist at Cardiff University in Wales, who helped discover NDM-1. But he adds that it's "only a matter of time before they pass their genes on to the nastier types of *E[scherichia] coli* and *Klebsiella*. And then we're in trouble."

With soil samples from Nova Scotia and elsewhere in hand, Wright and his team set out on a mission to find inhibitors of NDM-1. The researchers engineered a strain of *E. coli* to express the enzyme, grew the bacteria on plates containing the β-lactam meropenem, and added extracts derived from environmental microorganisms that laboratory members had collected on

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their holidays. Was there anything in the soil that could render bacteria susceptible again to the antibiotic?

The idea of looking to nature for drug leads has a long and proven track record in the fight against bacteria. Penicillin was discovered in the 1920s in a fungus; streptomycin in the 1940s in a bacterium. The approach may not have yielded any new broad-spectrum bacteria-killers for decades, but as Christian Melander, a chemist at North Carolina State University, points out: "We haven't looked for this kind of adjunctive activity a lot with natural products."

Hunting Down Inhibitors

As a first pass, Andrew King, a graduate student in Wright's laboratory, screened a small collection of 500 soil extracts. To everyone's surprise, King got a hit right off the bat. "I thought the screen had gone awry," recalls Wright. "We'd been screening tens of thousands of molecules for 15-plus years, and you never find something in the first 500."

But sure enough, from a strain of *Aspergillus* fungus collected in Kejimkujik, King had stumbled upon a molecule called aspergillomarasmine A (AMA). This fungal product neutralized NDM-1 and restored the killing power of meropenem, both in cultured NDM-1–expressing *E. coli* and in *Klebsiella*-infected mice.

To see how broad-acting AMA might be, King shipped off a sample to Walsh, who had amassed a global collection of more than 200 metallo-β-lactamase–expressing superbugs from Russia, India, Pakistan, Australia, North Africa, and South America. Among these laboratory-cultured strains, Walsh found that the compound suppressed resistance in 88% of NDM-1$^+$ bacteria and in 90% of bacteria with another metallo-β-lactamase called Verona integron-encoded metallo-β-lactamase, or VIM.

AMA is a potent inhibitor and, assuming that its safety profile is all good, it "has a place in the antimicrobial arena," says Walsh, a coauthor of the *Nature* study (1) that reported the compound's discovery.

Actually, as it turns out, it was more of a rediscovery. Chemists in France had first described AMA more than 50 years ago, detailing the compound's structure and its ability to wilt plant leaves. A Japanese drug company later investigated AMA as a possible treatment for high blood pressure.

Although King confesses he would have preferred to uncover something new, there was an upside to AMA's research history: the Japanese scientists had shown that AMA had a low-toxicity profile in mice. That was a relief, given AMA's mechanism of action, and it helped bolster King's confidence that the compound could prove safe in people.

Threading the Needle

AMA works by mopping up the zinc ions that metallo-β-lactamases normally use to crack open the β-lactam rings of antibiotics. But zinc has many other functions in the body, and too much zinc removal can lead to deadly side effects. "Threading that needle is where all the difficulty is," says Wright, who has also discovered other, more toxic zinc-binding adjuvants (3).

Wright and his colleagues recently described a method for synthesizing AMA from scratch (4), meaning they could start making derivatives as back-up drugs in case AMA doesn't pan out in the clinic. That's exciting to chemists like Melander, whose own research has demonstrated the adjuvant potential of a class of compounds known as 2-aminoimidazoles. A company Melander founded, called Agile Sciences, is now sorting through more than 700 derivatives for the best adjuvant candidates.

For now, though, Wright's team is sticking with AMA, and they're purifying huge amounts of it from *Aspergillus*. "The fungus just cranks this out quite happily," says King, who finished his doctorate earlier this year and is now a postdoctoral fellow at the Massachusetts Institute of Technology. At one point, King was growing so much fungus that he resorted to using leftover carboys from the water cooler when he ran out of conventional labware.

Hopefully the compound will have legs to keep moving further in development.

—Ann Eakin

The AMA then gets sent to the US National Institute of Allergy and Infectious Diseases (NIAID), which has spent about $800,000 running additional safety and efficacy studies. Through the institute's Concept Acceleration Program—a five-year-old program to help bring treatments for public health threats to market—NIAID has been able to show that AMA is nontoxic in dogs and that it restores sensitivity to meropenem in several mouse models of infection. Buoyed by the data thus far, Wright and others are drumming up investor interest in hopes of establishing a start-up based on the adjuvant.

But AMA isn't a sure thing. It's undergoing further preclinical evaluations and will then need to prove its worth in human clinical testing. Still, so far so good, reports Ann Eakin, a senior scientific officer at NIAID. "The data look promising," Eakin says. "Hopefully the compound will have legs to keep moving further in development."

1 King AM, et al. (2014) Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature* 510(7506):503–506.

2 Review on Antimicrobial Resistance (2014) Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. (HM Government, UK). Available at https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf.

3 Falconer SB, et al. (2015) Zinc chelation by a small-molecule adjuvant potentiates meropenem Activity in vivo against NDM-1-producing *Klebsiella pneumonia*. *ACS Infect Dis* 1(11):533–543.

4 Koteva K, King AM, Capretta A, Wright GD (2016) Total synthesis and activity of the metallo-β-lactamase inhibitor aspergillomarasmine A. *Angew Chem Int Ed Engl* 55(6):2210–2212.