ClpPing persistence

By Chris Cain, Senior Writer

Northeastern University researchers have combined traditional antibiotics with compounds that activate bacterial clpP protease and cured mice with severe, highly drug-resistant *Staphylococcus* biofilm infections.¹ **Arietis Corp.** is developing analogs of the compounds to treat persistent bacterial infections.

Bacterial persistence occurs when a subpopulation of bacteria slows its growth rate and becomes insensitive to growth-inhibiting antibiotics. One place where this commonly occurs is in biofilms, which are surface-attached bacterial communities held together by extracellular polymeric matrices.

In particular, some *Staphylococcus aureus* infections are notorious for their persistence in the face of antibiotics, including those associated with endocarditis, osteomyelitis and implanted medical devices.

Kim Lewis, a professor and director of the Antimicrobial Discovery Center at Northeastern, told *SciBX* that a longtime lack of success in developing effective treatments for persistent infections led his team to attack the problem from a different angle.

"We knew from years of work on persisters that pathways for their formation are highly redundant and that trying to find a compound that inhibited persister formation was not going to be productive—we effectively learned what not to do," he said.

"Persisters are dormant and in a metabolic state where established antibiotic targets are shut down, so antibiotics that inhibit essential proteins are not going to work," he continued. "That left us with one possibility—to look for something that is going to activate and corrupt an essential function without requiring energy.

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His team homed in on a class of molecules called acyldepsipeptides (ADEPs), which are natural products that kill bacteria by binding and activating the clpP protease, according to 2005 findings by **Bayer AG**.² ClpP is a key regulator of bacterial protein homeostasis that is conserved in most bacteria. Its activity is normally tightly regulated and requires an ATPase or another accessory protein to initiate proteolysis. ADEPs allow clpP to bypass these regulatory processes, resulting in uncontrolled proteolysis and ultimately cell death.

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To test whether ADEPs could help target persistence, Lewis' team incubated an ADEP analog called ADEP4 with *S. aureus* grown *in vitro* to stationary phase, in which the cells no longer divide and become metabolically inactive. Over a three-day treatment period, ADEP4 significantly reduced the number of viable bacteria, whereas the traditional antibiotics ciprofloxacin, vancomycin, linezolid and rifampicin had no effect.

Proteomic analyses confirmed that incubation with ADEP4 triggered extensive protein degradation in stationary phase bacteria.

When ADEP4 was combined with rifampicin, linezolid or ciprofloxacin, *S. aureus* was completely eliminated from the culture. This result was repeated when ADEP4 plus rifampicin was tested against a variety of *S. aureus* strains, including methicillin-resistant *S. aureus* (MRSA).

Finally, the group turned to a mouse model of severe *S. aureus* infection. In the model, mice are first treated with cyclophosphamide to suppress the immune system, and then a large dose of *S. aureus* is injected into the thigh and allowed to incubate for 24 hours before antibiotic treatment.

ADEP4 plus rifampicin completely eliminated infection in all five mice, whereas animals treated with rifampicin, vancomycin or a combination of the two had reduced numbers of bacteria but did not clear the infection.

Results were published in *Nature*.

Resistance questions

Jason Sello, an associate professor of chemistry at **Brown University**, noted that the results highlight a conceptually new approach to fighting persistent bacterial infections.

"The findings are indeed quite remarkable. The reported observations indicate that metabolically quiescent yet imminently lethal bacteria can be killed by activation of endogenous enzymes that degrade macromolecules," he said.

Sello's group has developed inhibitors of clpP that kill *Mycobacterium tuberculosis*³ and analogs of the ADEP activators of clpP that have enhanced pharmacological properties and are active against *S. aureus*, *Streptococcus pneumoniae* and Enterococci. ClpP is an essential gene in *M. tuberculosis*.

That is not the case in many bacterial species, including *S. aureus*, in which, unlike the essential protein complexes inhibited by traditional antibiotics, clpP is not essential for bacterial growth *in vitro*. This means it is relatively common for strains to develop resistance to ADEPs *in vitro* by acquiring inactivating mutations in clpP.

Thus, an important next question for ADEPs and other clpP-targeting compounds is whether the development of resistance can be overcome by combination strategies.

Scott Gray-Owen, a professor of molecular genetics at the **University of Toronto**, is not convinced that the resistance often seen *in vitro* means

it will develop *in vivo*. “Just because we can knock the gene out and the bacteria can grow *in vitro*, it does not mean that the bacteria will necessarily be infectious anymore. The ‘essential’ nature of genes is often

defined by growth in extremely rich media in the lab and may not represent their role in the host,” he said.

Gray-Owen is collaborating with Walid Houry, a professor of biochemistry at the University of Toronto, to develop compounds that activate clpP, including molecules specific for particular bacterial strains.⁴

Lewis said that *clpP*-mutant *S. aureus* is highly attenuated, and in the *Nature* paper his team showed that the mutants were more sensitive to traditional antibiotics than wild-type cells. He also noted that if an entire bacterial population, including persisters, is rapidly wiped out by combination treatment, there would be less time for resistance to emerge.

Houry agreed. “If this approach is working on the dormant bacteria that are residing in the background, if you kill them off in the first pass before they begin to grow, then resistance may not emerge,” he said.

Another question is whether activating clpP could help treat persistent Gram-negative infections, such as *Pseudomonas aeruginosa*. Lewis said that ADEPs do not effectively penetrate the membrane of Gram-negative bacteria, so distinct compounds would need to be developed to test that hypothesis.

Prabhavathi Fernandes, founder, president and CEO of **Cempra Inc.**, was enthusiastic about the findings but wanted to see more characterization of ADEP4, including resistance frequency and pharmacokinetic data, and particularly more data on the safety profile of the ADEPs after longer-term dosing studies. “The results are thought provoking and certainly remarkable. There is an urgent need for antibiotics that can overcome biofilm infections,” she said. “It’s early but beautiful work.”

She added that pharmacokinetics would be particularly important if the drug were to be used in combination with other antibiotics. “Both drugs have to be in the right place at the right time to prevent the resistance; if one goes away you get resistance. In a sense each drug is protecting the other,” she said.

Fernandes and Sello both said that they would like to see the compound tested in combination with other commonly used classes of antibiotics, including β -lactams with β -lactamase (LACTB) inhibitors.

Cempra’s solithromycin, a macrolide antibiotic, is in Phase III testing for community-acquired bacterial pneumonia (CABP). The

company’s Taksta (fusidic acid) has completed Phase II trials for acute bacterial skin and skin structure infections (ABSSSIs) and is in Phase II testing to treat prosthetic joint infections, in which it has received orphan designation.

Arietis, an antibiotic company founded by Lewis in 2008, is generating pharmacology and safety data for ADEPs and has exclusively licensed patents covering analogs and their combination with traditional antibiotics from Northeastern University.

“We are performing target validation studies on clpP and actively looking for other antimicrobials that function by activating a cellular process,” said COO Michael LaFleur. “We are also performing preclinical pharmacokinetic, pharmacodynamic and safety assessments on ADEPs and are working with a medicinal chemistry group on analogs of ADEP with improved pharmacological properties.”

LaFleur said that the company has received more than \$6 million in Small Business Innovation Research (SBIR) grants since 2008 to fund its research.

Arietis plans to identify compounds that can kill bacteria by activating or corrupting the function of additional targets, and Lewis’ lab is continuing to study mechanisms of persistence.

Gray-Owen said that other bacterial proteases could be attractive candidates to activate to kill persistent bacteria. However, he said that proof of concept for activating clpP was only possible because of years of basic work in understanding how ADEPs kill bacteria.

“This absolutely rationalizes basic biology research; no one would have thought to rationally design a compound with this mechanism of action. It would be hard to predict that dysregulating this complex would kill bacteria. That is why it had to emerge from basic biological insights into the mechanism of ADEP killing,” he said.

Cain, C. *SciBX* 6(47); doi:10.1038/scibx.2013.1338
Published online Dec. 12, 2013

REFERENCES

1. Conlon, B.P. *et al. Nature*; published online Nov. 13, 2013; doi:10.1038/nature12790
Contact: Kim Lewis, Northeastern University, Boston, Mass.
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2. Brötz-Oesterhelt, H. *et al. Nat. Med.* **11**, 1082–1087 (2005)
3. Compton, C.L. *et al. ACS Chem. Biol.*; published online Sept. 18, 2013; doi:10.1021/cb400577b
4. Leung, E. *et al. Chem. Biol.* **18**, 1167–1178 (2011)

COMPANIES AND INSTITUTIONS MENTIONED

Arietis Corp., Boston, Mass.
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