Stop talking at the back

Martina Habeck, freelance writer

A new compound has been shown to prevent communication among *Pseudomonas aeruginosa*. This research has the potential to lead to a new class of antibiotics.

P. aeruginosa infections rarely trouble healthy individuals. However, the bacteria thrive in the lungs of cystic fibrosis patients, and they also target immunocompromized patients and burn victims – they are also extremely difficult to treat. Not only are these bacteria resistant to most of the antibiotics currently available but they can also produce a slimy biofilm that coats surfaces and shields the bacterial community from the few antibiotics that would otherwise still work.

At present, these bugs can not be killed; therefore, scientists are looking for other ways to keep them at bay. A tempting approach is to block the production of virulence factors and biofilm formation by interfering with quorum sensing.

Strength in numbers

Quorum sensing systems enable pathogens to measure their own density and launch an attack on host tissues as a large population, rather than as individual cells. The bacteria emit a signal molecule (autoinducer) that, at low cell density, diffuses into the surrounding media. But with increasing cell density, the concentration of the autoinducer rises as well. When a threshold concentration is reached, the autoinducer binds to an intracellular receptor molecule that, when complexed with the autoinducer, acts as a transcriptional activator. Among other things, this mechanism helps to set off biofilm formation and the production of virulence factors.

P. aeruginosa has two quorum-sensing circuits, the *las* system and the *rhl* system. The *las* system consists of autoinducer synthase LasI, binding protein LasR and autoinducer AI1; it controls the production of LasB elastase and other virulence factors and is required for the activation of the second quorum sensing system (*rhl*).

A new class of antibiotics?

Although some researchers remain sceptical as to whether a new class of antibiotics can be found by targeting quorum-sensing systems, others have embraced the idea with enthusiasm. 'The exciting thing is that there has been talk for about a decade of trying to develop antivirulence drugs, rather than antibacterial drugs that kill bacteria,' says Everett Greenberg at the University of Iowa (http://www.uiowa.edu), one of the pioneers of quorum sensing research in *P. aeruginosa*. 'I think these quorum sensing signals represent a real opportunity to test the idea, because we know they are important in controlling virulence.'

Several academic groups and a few companies are pursuing various strategies to interfere with quorum sensing. In the commercial arena, Vertex (http://www.vpharm.com) are screening huge libraries of small-molecule compounds that might do the trick, and Biosignal (http://www.biosignal.com.au) are working with furanones, which are natural quorum-sensing inhibitors emitted by a seaweed to prevent bacterial growth on its surface.

Other groups have tried to rationally design autoinducer analogues that block quorum sensing. The signal molecules of Gram-negative bacteria are homoserine lactone (HSL)-based molecules that differ in their acyl side chains. Previous efforts have centred on altering this acyl structure, but this approach did not deliver potent antagonists. Therefore, Hiroaki Suga and colleagues at the University of Buffalo (http://www.buffalo.edu) decided to tackle the HSL group. Suga believes that it is this structure that is actually important for R protein activation.

Breaking the code

To explore this hypothesis, Suga and colleagues synthesized a library of 96 AI1 analogues in which the HSL group is replaced by amines or alcohols. To screen for biological activity, they used a *P. aeruginosa* strain in which *lasI* and *rhlI* were knocked out so that the bacteria could not produce any autoinducers. In addition, the bacteria carried a GFP reporter gene under the control of the *lasI* promoter. Using this approach, they found an agonist that was as potent as AI1.

Based on the structure of this agonist, they designed three other compounds that were similar in structure and again screened for agonist activity. They were hoping that this experiment would tell them details about the structural elements required for R protein activation.

But they were in for a surprise: the reporter protein remained silent when the strains were exposed to compound 3 [1]. 'We could not believe that this molecule failed to bind to the target protein, because the structure we changed is really tiny,' recalls Suga.

He suspects that compound 3 acts as a competitive inhibitor, and results

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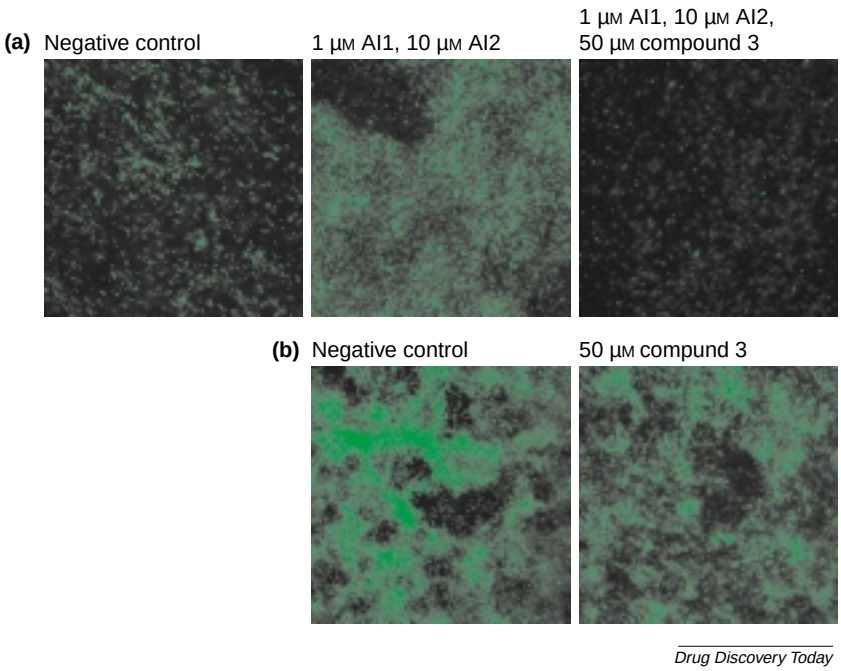

Figure 1. Effect of compounds on biofilm formation of *Pseudomonas aeruginosa*; the bacteria, which express the green fluorescent protein, are grown on cover slips in a static culture. When grown in the presence of autoinducers, the bacteria form a biofilm. The addition of compound 3 blocks biofilm formation. **(a)** *P. aeruginosa* PAO-JP2 (laboratory strain). **(b)** *P. aeruginosa* PAO-1 (wildtype strain). Figure reproduced, with permission, from Ref. [1].

of an antagonist assay support this hypothesis. Other assays revealed that compound 3 blocks the production of various virulence factors and prevents biofilm formation (Fig. 1).

Greenberg believes the result is promising. However, he is concerned that this compound itself might not be useful as a drug: 'It is too weak an inhibitor'. Indeed, compound 3 inhibited *lasI* induction by only 35%. 'But it may be possible to further develop it into a drug,' reckons Greenberg.

According to Suga, this won't be too difficult. 'The approach we used is very flexible, and it is possible to expand it by working with other focused libraries. Therefore, we can look for more potent antagonists in the future.'

Reference

1 Smith, K.M. *et al.* (2003) Induction and inhibition of *Pseudomonas aeruginosa* quorum sensing by synthetic autoinducer analogs. *Chem. Biol.* 10, 81–89

Centenarians provide genetic clue to age-related disease

Sabine Louët, freelance writer

Studies on people who have passed their 100th birthday are revealing the role of genetics in age-related disease. Many age-related diseases result from inflammatory processes, and centenarians enjoy unusually low inflammatory profiles, says Italian immunologist, Claudio Franceschi, Professor of Immunology at the University of Bologna, Italy (http://www.unibo.it).

Link between inflammation and ageing

Franceschi, says that his work on centenarians has revealed a genetic link between a mechanism for chronic inflammation and ageing. 'People are prone to develop inflammation on a genetic basis.'

Franceschi discovered that centenarians present low levels of the pro-inflammatory cytokine interleukin 6 (IL-6) and high levels of the anti-inflammatory cytokine IL-10. High levels of IL-6, which is produced in muscles and bones, is related to loss of muscle mass and power with age. Such status translates into frailty and disability and the occurrence of diseases, such as osteoporosis.

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