News & views

Microbiology

New antibiotic targets a drug-resistant bacterium

Morgan K. Gugger & Paul J. Hergenrother

Infections caused by drug-resistant strains of the bacterium *Acinetobacter baumannii* have been hard to treat in the clinic. A new class of antibiotics has been identified with the potential to tackle these microbes. **See p.566 & p.572**

Drug-resistant bacterial infections are a looming threat to global public health and increasingly contribute to the death of infected individuals[1]. Particularly concerning are illnesses caused by 'Gram-negative' bacteria, because these microbes are encased in both inner and outer membranes that are challenging for most antibiotics to cross[2,3]. An antibiotic-resistant Gram-negative bacterium called carbapenem-resistant *Acinetobacter baumannii* (CRAB) is particularly difficult to treat, and the US Food and Drug Administration (FDA) has not approved any new classes of antibiotic for harmful Gram-negative bacteria in more than 50 years. Treatment options for CRAB infections continue to dwindle as mortality rates are rising, with some estimated death rates reaching approximately 50% for invasive infections[4]. On pages 566 and 572, respectively, Zampaloni *et al.*[5] and Pahil *et al.*[6] report progress in the effort to develop much-needed treatments.

Many antibiotics approved for clinical use are small-molecule drugs (which typically have a molecular weight of less than 600 daltons) that engage targets in the bacterial cytoplasm after crossing the outer and inner membranes. These antibiotics are often derivatives of existing classes; the identification of truly new classes of drug and their advancement to clinical trials are exceedingly rare[7].

To make progress on this front of antibiotic discovery, Zampaloni *et al.* identified members of a unique chemical class that initially possessed only a glimmer of antibacterial activity. The authors examined a collection of approximately 45,000 molecules called tethered macrocyclic peptides (MCPs). These have molecular weights of approximately 800 daltons, which is larger than that of most antibiotics.

Zampaloni and colleagues identified an MCP that selectively kills *A. baumannii*. This compound was further optimized for efficacy and tolerability, using results from a new type of test (one based on blood-plasma compatibility) as a key part of the decision-making process to determine whether the drug should advance to the next stage. This fine-tuning culminated in zosurabalpin, a drug candidate with a charge-balanced nature that contributes to notable improvements in solubility and safety compared with the profiles of the other molecules that the authors identified.

Copious evidence provided by Zampaloni *et al.* indicates that zosurabalpin kills *A. baumannii* through a previously unknown mode of action, striking a target that no FDA-approved drugs hit. Zosurabalpin inhibits a key process, transport of the molecule lipopolysaccharide (LPS), by inhibiting a complex of proteins called $LptB_2FGC$. This complex is essential for transporting LPS to the bacterial surface to create the outer-membrane structure of Gram-negative bacteria. Inhibition of this transport causes LPS to build up to toxic levels inside the cell, eventually leading to cell death (Fig. 1).

Pahil and colleagues present structures (obtained using an X-ray technique) revealing that zosurabalpin engages $LptB_2FGC$ only when the complex is bound to LPS, indicating that the presence of LPS is essential for the mode of action of this drug. Moreover, the basis of specificity for *A. baumannii* (zosurabalpin is not effective against other bacteria) lies in this protein complex: amino-acid sequences of the Lpt proteins that form the complex have poor evolutionary conservation among different groups (genera) of bacteria. This previously unknown mode of antibiotic action suggests that pre-existing resistance is unlikely.

Zosurabalpin seems to have considerable potential for use in the clinic. It was effective against more than 100 CRAB clinical samples tested in the laboratory, and it considerably reduced the levels of bacteria in mice with CRAB-induced pneumonia and prevented the death of mice with a CRAB-induced abnormal immune response called sepsis. Building on this demonstrated safety, tolerability and

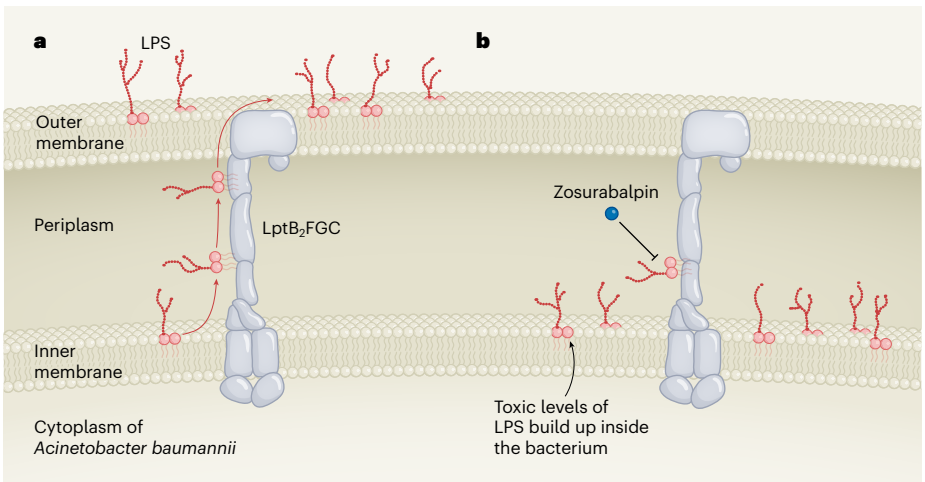

Figure 1 | A new type of antibiotic. Zampaloni *et al.*[5] and Pahil *et al.*[6] report the identification and analysis of an antibiotic called zosurabalpin that can kill the bacterium *Acinetobacter baumannii*. Antibiotic-resistant strains of this bacterium are hard to treat in the clinic. **a**, The complex of proteins called $LptB_2FGC$ is located between the microbe's inner and outer membrane layers in a region called the periplasm. $LptB_2FGC$ transports a type of molecule called a lipopolysaccharide (LPS) from the inner to the outer membrane. **b**, Zosurabalpin blocks LPS transport, and the abnormal build-up of LPS in the cell kills the bacterium.

efficacy, zosurabalpin has been evaluated in two phase I clinical trials[8].

Compounds isolated from nature or the close relatives of such products have long dominated antibacterial drug discovery, because many such compounds have evolved the ability to kill bacteria. Indeed, only a handful of antibiotic classes — most notably sulfonamides, fluoroquinolones and oxazolidinones — are synthetic[3]. As such, the discovery and development of zosurabalpin is particularly notable, and it demonstrates that examining alternative sources of possible antibiotics beyond those that are usually tested — including compounds from sources such as the MCP collection, which have a higher molecular weight than is typical for antibiotics — can be fruitful. This has also been demonstrated in other work with DNA-encoded molecular 'libraries'[9].

Drug discovery that targets harmful Gram-negative bacteria is a long-standing challenge owing to difficulties in getting molecules to cross the bacterial membranes to reach targets in the cytoplasm. Successful compounds typically must possess a certain combination of chemical characteristics[10,11]. It therefore makes sense to pursue antibiotics that engage targets on the outside of the cell or in the space between the inner and outer membranes, termed the periplasm. $LptB_2FGC$ resides in the periplasm, so zosurabalpin does not need to reach the bacterial cytoplasm.

This success with zosurabalpin mirrors that of other newly discovered antibiotics, such as darobactin[12] and dynobactin[13], which are also high-molecular-weight compounds that need to reach targets outside the cytoplasm. Non-cytoplasmic targets can probably be engaged by more-chemically-diverse compounds (with respect to their size and shape) than can cytoplasmic targets.

With nearly all antibiotics, mutations (often in the target) arise that lead to antibiotic resistance. Indeed, mutations in genes encoding components of $LptB_2FGC$ in zosurabalpin-resistant CRAB identified in the lab result in a striking decrease in zosurabalpin's ability to kill the bacterium. In one case, a single-nucleotide mutation in the sequence encoding the $LptB_2FGC$ complex resulted in a more than 256-fold decrease in antibiotic activity. The unusual mode of action of zosurabalpin requires LPS, and this foreshadows another potential limitation because *A. baumannii* is unusual in that it does not need LPS to be viable[14]. The bacterium can halt LPS synthesis if necessary for survival — potentially rendering zosurabalpin ineffective against LPS-deficient *A. baumannii*. However, such a change would attenuate bacterial virulence[15], and it remains to be determined whether this type of antibiotic-resistance mechanism will be observed in the clinic.

This discovery opens the door to targeting the LPS-transport system of other problematic Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. In addition, there is now an appreciation that disruption of normal gut microbes (the microbiome) is deleterious to human health; such a disturbance is a consequence of essentially all antibiotics used in the clinic. Given zosurabalpin's high specificity for *A. baumannii*, it might be a microbiome-sparing antibiotic. The movement towards bacterium-specific antibiotics is a new development, and one that can be facilitated by diagnostics that can rapidly identify specific harmful bacteria in infected individuals[3]. Given that zosurabalpin is already being tested in clinical trials, the future looks promising, with the possibility of a new antibiotic class being finally on the horizon for invasive CRAB infections.

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The authors declare no competing interests.
This article was published online on 3 January 2024.

Computer science

Large language models help programs to evolve

Jean-Baptiste Mouret

A branch of computer science known as genetic programming has been given a boost with the application of large language models that are trained on the combined intuition of the world's programmers. **See p.468**

Although machines outperform humans at many tasks, from manufacturing to playing games[1,2], they are commonly considered incapable of creating or inventing things. But this is changing: over the past three years, technology companies have been releasing artificial intelligence (AI) programs that can draw or write with impressive creativity. Scientific discovery might be the next target, as computers start to uncover knowledge that has eluded scientists. On page 468, Romera-Paredes *et al.*[3] report an autonomous mathematical discovery for which AI was used — not to check a proof or to execute tedious computations, but to solve open problems. This proof of concept is likely to be followed by other programs like it, as software becomes a creative contributor to scientific discoveries.

Romera-Paredes and colleagues' work is the latest step in a long line of research that attempts to create programs automatically by taking inspiration from biological evolution, a field called genetic programming[4]. The process starts with running many random programs to find out how well each one can solve a target problem. The best programs are then selected, copied and randomly modified, in a manner that is similar to genetic variation. The process then begins again with these modified programs, which are selected and modified until one program solves the problem adequately.

The key question in genetic programming is how to represent programs so that they can be modified easily, but meaningfully, by random variation. In other words, what is the 'DNA' of a computer program? For instance, adding random letters to a program written in the Python scripting language is unlikely to result in a program that follows Python syntax, which means that the vast majority of modified programs cannot be executed by the computer, and are therefore useless.

To approach this problem, genetic-programming researchers have taken inspiration from compilers, which are programs that transform text written in a programming language into code that a computer can