PERSPECTIVE

The age of the phage

It's time to use viruses that kill bacteria again, say **Shigenobu Matsuzaki**, **Jumpei Uchiyama**, **Iyo Takemura-Uchiyama** and **Masanori Daibata**.

Bacteriophages, or simply 'phages', are viruses that infect and in some cases destroy bacterial cells. Scientists started using phages as a medical therapy in the early 1900s. However, the technique fell out of favour in the 1940s, largely due to the introduction of antibiotics, which provided protection against a broader range of infections.

But as the problem of antibiotic resistance increases, more countries are revisiting phage therapy. Last year, for instance, the European Union (EU) funded a project called Phagoburn to explore the use of phage therapy to treat burn wounds infected with bacteria. Phagoburn involves institutions and hospitals in Belgium, France and Switzerland. The EU hopes the results from this project can be used, the project's website says, "for an optimization of current regulatory guidelines in phage therapy", because no new forms have been approved recently.

Countries that aim to introduce phage therapy will need to prepare their own guidelines for approving it, including methods for phage selection, preparation and administration. But recent advances in phage therapy suggest that such regulatory efforts would bring big rewards in treating bacterial infections.

AVOIDING IMMUNITY

Phages are a natural part of the microbial ecosystem. Environments such as sea water, fresh water and soil all contain millions of phage species. Different phage species are specific to particular bacterial species, and they can infect bacteria without harming animal or plant cells.

When faced with a bacterial infection, scientists first isolate candidate phages from the environment. The bacteria can be treated with a sample of water that naturally contains phages. If the bacteria die, the sample can be centrifuged, leaving the phages at the top to be collected and tested to see which ones killed the bacteria. Either the phage or its products, such as bacteriolytic enzymes called endolysins, can then be used as antibacterial agents in pills and ointments, often requiring just a single dose.

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However, despite early success, phage therapy was largely abandoned when antibiotics came along, and is used today in only a few countries, including Russia, Georgia and Poland. Phage therapy declined in part because it focuses on treating specific infections, rather than on treating a range of bacteria. Some studies concluded that it failed because highly specific phages were simply tested against the wrong bacteria.

Yet phage therapy has several advantages over using antibiotics. First, because the bactericidal mechanism is completely different from the way antibiotics work, it is effective against multidrug-resistant bacterial infections. Second, phage therapy is highly species specific, meaning it is unlikely to change the bacterial flora of a patient and cause gastrointestinal side effects. Third, the propensity of phages to proliferate allows the use of very low doses.

Opponents of phage therapy often raise two potential problems: the appearance of phage-resistant mutant bacterial strains, and adverse reactions caused by the host's immune system against the phage. Modern techniques make it possible to address both of these concerns, however. First, using a cocktail of several different phages, or the advance preparation of mutant phages, overcomes any issue of bacterial resistance. Second, to stop phage therapy activating someone's immune system, a medical treatment can use phages with innate characteristics that are unlikely to elicit an immune response, use mutant phages that are not recognized by the immune system, or use some combination of the two. If a phage does somehow turn on the immune system, it can be treated with polyethylene glycol, for example, which will reduce the immune response.

Some phages can also produce toxins, but there is a way of resolving that problem too. Modern high-throughput techniques have moved phage therapy beyond screening water samples for potential treatments. Next-generation sequencing, for example, allows genomic DNA sequences from multiple phages to be analysed simultaneously. This makes it easier to detect suitable candidates for phage therapy that lack harmful genes, such as those that produce toxins or drug resistance.

THE SILK ROAD

In addition, antibacterial research, like all drug discovery, benefits from lower-cost approaches. Modern drug development programmes often include studies on rodents, which require extensive experimental facilities and carry high experimental costs. For phage therapy, a less expensive invertebrate-based platform could be used. Kazuhisa Sekimizu and his colleagues at the University of Tokyo, for example, showed that silkworm larvae and mice provide comparable results regarding the effectiveness of experimental antibiotic therapies[1,2].

We have used silkworm larvae to test phage therapy against *Staphylococcus aureus* infections. Using two new phages to infect the bacterium, we found no adverse effects on the silkworm, but the phages did destroy the bacteria cells[3]. Our results using silkworm larvae were similar to those using these phages against *S. aureus* infections in mice.

Although we continue to obtain benefits from antibiotics, as we did in the twentieth century, the problems of antibiotic-resistant bacteria are set to increase, making it unlikely that antibiotics will remain effective forever. The latest techniques make it easier and faster to find phages to fight specific bacteria, and with less risk of resistance than using antibiotics. Phage therapy may be from a bygone era, but these advantages make today the age of the phage. ■

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