

INFECTIOUS DISEASE

Faster drugs for unknown bugs



Emerging infectious diseases pose the threat of a looming global pandemic. As illustrated by the outbreak of the SARS coronavirus (SCV) in 2003, there is a pressing need for new effective treatments that can be rapidly developed in response to previously unknown viruses. Reporting in *Nature Medicine*, Li *et al.* took a step towards this goal by demonstrating that intranasal delivery of SARS-specific small interfering RNA (siRNA) was effective against SARS in a rhesus macaque model — providing hope that siRNA could provide the means to move rapidly from gene sequence to targeted therapeutics for many previously intractable diseases.

The recently established rhesus macaque SARS model, based on intranasal instillation of a highly virulent SARS strain, mirrors the sequence of pathologies in humans with SARS. Taking advantage of previously identified siRNA sequences that have a synergistic anti-SCV effect *in vitro*, the authors used a clinically approved aqueous carrier solution (D5W) to intranasally administer the therapeutic siRNA either 4 hours before, concurrently or 4 hours after infection of the animals with SCV. The effectiveness of this treatment was evident as

macaques from all three treatment regimes had a lower increase in body temperature, reduced viral loads and a reduced level of lung damage, indicating potent suppression of SCV-induced SARS pathology compared with SCV-infected animals that received no siRNA or unrelated siRNAs.

These results might be attributable to a protective effect conferred by siRNA on cells against SCV infection (the prophylactic treatment could even lead to direct degradation of viral RNA by pre-existing siRNA in the upper airway tract upon entry of the viral particle); the inhibition of viral protein synthesis in infected cells caused by the degradation of SCV mRNA; or preventing the replication of the SCV genome and the spread of virions to uninfected cells. As neutralizing antibodies were also detected soon after infection, the authors speculated that several antiviral mechanisms were operative.

Importantly, the effect of the siRNA did not seem to be caused by the induction of a pro-inflammatory interferon response, which can potentially exacerbate symptoms and lung damage. Specific emphasis was placed on tolerability and safety — as

ANTIMICROBIAL DRUGS

Accept some substitutes

A new high-throughput method for synthesizing and screening peptides for antimicrobial activity has been used to identify novel broad-spectrum antibiotic candidates. Robert Hancock and colleagues describe in *Nature Biotechnology* the optimization of a bovine antibacterial peptide by amino-acid substitution. Combining favourable substitutions generated small peptides with activity against several common microbes, including *Escherichia coli* and *Staphylococcus aureus*. The authors estimate that with the assistance of two robots, 100,000 single peptides could be synthesized and screened per year using this method.

Novel antibiotics are urgently needed to address the rapid emergence of drug resistance among Gram-positive bacteria such as multidrug-resistant *S. aureus* and vancomycin-resistant enterococci, against which most new classes of antibiotic are largely ineffective. However, the recent worrying isolation of Gram-negative ‘superbugs’, such as multi-drug resistant *Pseudomonas* strains, suggests that the

development of broad-spectrum antibiotics would be desirable. Cationic antimicrobial peptides have the advantage that they often kill both Gram-positive and -negative bacteria, including clinically common drug-resistant strains, and they have also been shown to activate host innate immunity and ward off sepsis.

The authors used a variant of a bovine cationic peptide called Bac2A — a small, linear peptide with moderate activity against both Gram-negative and -positive bacteria — as a starting point for synthesizing a library of peptides containing every possible amino-acid substitution on a cellulose support. This method of peptide synthesis is very high-throughput, but the amount of peptide generated is not enough to use in conventional assays for establishing the minimal inhibitory concentration (MIC) of antimicrobials. The authors therefore developed a more sensitive assay to evaluate antimicrobial activity. They created a strain of *Pseudomonas aeruginosa* containing a luciferase reporter gene incorporated into a constitutively active gene involved in ATP-dependent flagellar biosynthesis. So long as the bacterium produces ATP, the flagellum is synthesized and light is produced that can be quantified; a reduction in luminescence therefore reflects a decrease in ATP production caused by antimicrobial action of the peptide.

The authors used this screen to evaluate their substitution library and found 46 peptides that had improved activity compared with Bac2A, a finding confirmed by screening using a conventional MIC assay. Multiple substitutions, based on data revealing the relationship of certain amino acids to antimicrobial activity, were found to further improve potency; in particular, variants with three and five favourable substitutions had excellent broad-spectrum activity. Additional optimization to reduce the length of the peptides resulted in an 8-mer peptide, Bac8c, which was particularly effective at killing Gram-positive bacteria and *E. coli*, and could be a candidate for further investigation.

In addition to identifying new antibiotic candidates, this study describes methodology that enables the screening of novel antimicrobial peptides in a high-throughput manner that has not previously been possible using conventional assays. Moreover, in demonstrating its use the authors have revealed much about the role of specific amino acids in antimicrobial activity that could be useful for future drug discovery efforts.

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 **References and links**

ORIGINAL RESEARCH PAPER Hilpert, K. *et al.* High-throughput generation of small antibacterial peptides with improved activity. *Nature Biotechnol.* **23**, 1008–1012 (2005)