ANTIBACTERIAL DRUGS

StAMPing out antibiotic-resistant bacteria

Cationic α-helical antimicrobial peptides (AMPs) hold great promise for the treatment of multidrug-resistant (MDR) bacteria, which are unlikely to develop resistance to membrane lysis, the mechanism of action of such AMPs. However, linear AMPs are typically unstructured in solution, proteolytically labile and can non-specifically lyse mammalian cells. A new study now reports the design of more stable, membrane-selective AMPs termed stapled AMPs (StAMPs; α-helical AMPs stabilized using hydrocarbon linkers), which can kill antibiotic-resistant bacteria in mice.

To determine the optimal placement of the staple, the authors tested the lytic activity of a 58-member staple-scanning library of the 23-residue AMP magainin II (Mag2; from the African clawed frog, *Xenopus laevis*) against bacteria and red blood cells (RBCs; a model for lysis of mammalian cells). Staple placement that expanded the continuity of the hydrophobic landscape on the hydrophobic face of the amphipathic helix, regardless of the change in overall hydrophobicity, resulted in increased haemolytic activity. These insights were used to design an algorithm for creating StAMPs that selectively target bacterial membranes while minimizing haemolysis, expressed as a lyticity index (LI).

To dissect the mechanism of membrane selectivity, the authors performed hydrogen–deuterium exchange mass spectrometry (HX-MS) to probe the solvent accessibility of various Mag2 variants when in association with either anionic or zwitterionic liposomes, which model bacterial and mammalian membranes, respectively. Interestingly, a bacterial-membrane-selective Mag2 variant was inaccessible when incubated with anionic liposomes but not with zwitterionic liposomes, whereas a more indiscriminate variant was less accessible, irrespective of liposome type. These results suggest that the preferential interaction propensity of bacterial-selective Mag2 variants for negatively charged membranes plays a significant role in determining their lytic specificity.

AMPs can form pores in membranes by, amongst others, barrel-stave, carpet or toroidal pore mechanisms. To determine how StAMPs form membrane pores, the interaction of Mag2 variants with anionic or zwitterionic bilayers was probed. Quartz crystal microbalance (QCM) analysis revealed that a bacterial-selective variant interacted with the membrane in a mostly transmembrane orientation and thus likely forms pores by a barrel-stave or toroidal pore mechanism. This mechanism further contributed to membrane selectivity, as the presence of cholesterol, commonly found in mammalian but not bacterial membranes, inhibited membrane insertion.

The authors used their algorithm and mechanistic insights to design a clinically effective StAMP. To further stabilize the single-stapled Mag2 variant, they screened a staple-scanning library for double-stapled variants with no detectable RBC lysis but greater potency against Gram-negative bacteria than the single-stapled variant. HX-MS analysis showed that the candidate with the lowest LI had strong interaction selectivity for anionic liposomes and QCM results were indicative of transmembrane insertion. This candidate was highly effective in killing various MDR-resistant Gram-negative bacteria, with no resistance developing during one month of serial passaging.

> “StAMPs … can kill antibiotic-resistant bacteria in mice”

Finally, the efficacy of this double-stapled Mag2 variant was tested in vivo. Intravenous administration in mice revealed that the peptide had no effect on RBCs or liver or kidney parameters, although histological analysis revealed mild renal tubular degeneration in some mice. To address this problem, the authors introduced mutations predicted to reduce renal toxicity and confirmed that the modifications were effective by testing in vitro toxicity in human renal proximal tubular epithelial cells and, after repeated testing in vivo, kidney histology was found to be normal. In a mouse peritonitis-sepsis model, 75% of immunocompromised mice infected with MDR-resistant *Acinetobacter baumannii* treated with the optimized, double-stapled Mag2 peptide survived, whereas 75% of mice treated with the natural Mag2 died after 24 h and all untreated mice died within 12 h. Importantly, the predictive algorithm was capable of generating highly optimized candidate StAMPs for preclinical development from AMPs originating from disparate organisms and with diverse sequences.

Overall, these findings demonstrate the therapeutic potential of StAMPs for the treatment of antibiotic-resistant bacteria.

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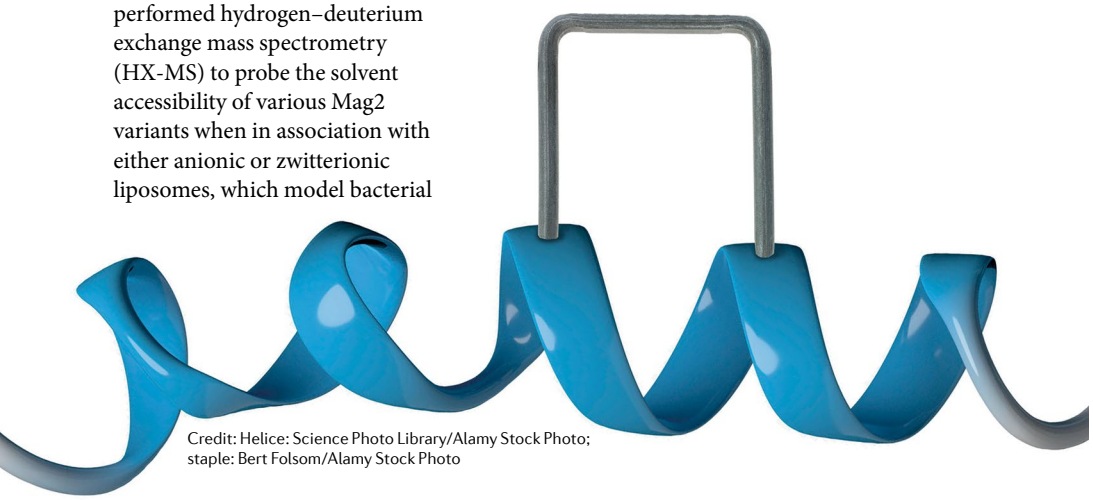

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