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Abstract: 93O

The synergy of a Lactoferrin-derived peptide with the antifungal drug Amphotericin B is lipid-mediated

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Invasive fungal infections (IFIs) are an underappreciated public health threat and particularly affect immunocompromised people. In certain health settings, IFIs show a mortality rate of 20-80%. Amphotericin B (AmB) is one of the most effective anti-fungal drugs but the drug's dose-limiting toxicity causes severe and chronic side effects. AmB targets and binds to ergosterol in fungal membranes, subsequently sequestering ergosterol and permeabilising the membrane. Due to the structural similarity of ergosterol and cholesterol, AmB also binds to cholesterol in mammalian membranes causing cytotoxicity.

Lactofungin (LFG) is a 30-residue peptide derived from the milk protein Lactoferrin. LFG is non-toxic to mammalian cells and exhibits no anti-fungal activity(1). Yet, LFG is highly synergistic with AmB on clinically relevant fungal strains, reducing the AmB dose up to 8-fold(1). Here, we investigated whether the synergy of LFG and AmB is a sterol-mediated mechanism. Membrane models containing 10%, 20% or 30% cholesterol or ergosterol were used to mimic mammalian and fungal cell membranes, respectively.

Data from RH421 fluorescence spectroscopy, measuring a change in membrane dipole, shows that addition of LFG to sterol-containing membranes does not change the dipole moment, implying that LFG does not bind to lipid membranes. This is supported by data from MD simulations, which indicate that LFG does not alter bilayer structure bilayer or the distribution of sterols in the bilayer.

Electrical impedance spectroscopy (EIS) data from tethered bilayer lipid membranes (tBLM) shows that LFG increases membrane-disruptive activity of AmB 2-4-fold for membranes containing ergosterol, the main sterol found in fugal cell membranes. This synergy is not observed for membranes containing cholesterol, the main sterol found in mammalian cell membranes. The selectivity of the LFG synergy with AmB for ergosterol over cholesterol indirectly increases the specificity of AmB.

Our data suggests that the synergy of LFG and AmB is ergosterol-specific, demonstrating the potential of LFG as an adjuvant to AmB treatments. While LFG may decrease the cytotoxicity and increase the fungicidal effect of AmB, further studies to elucidate on the mechanism of synergy are needed.

1 Fernandes KE, Payne RJ, Carter DA. Lactoferrin-derived peptide Lactofungin is potently synergistic with Amphotericin B. *Antimicrob. Agents Chemother.* 2020;**64**(10):e00842-20.