Ultrashort Lipopeptide C12-OOWW-NH_2 as an Antifungal Agent to Treat Fungal Keratitis

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Background:

In recent decades there has been a global increase in the prevalence of sight-threatening microbial corneal infections [1], specifically of fungal keratitis (FK), which is characterised by severe inflammation and ulceration of the cornea.

Polyene antifungal agents (e.g., amphotericin B) constitute the current treatment strategy for FK, however, their treatment efficacy is limited due to several pharmacological factors including poor drug stability and large molecular size. More recently, antimicrobial peptides have been investigated in treating ocular fungal infections, but face limitations regarding ocular toxicity and cost of production. Peptido-mimetics have therefore been developed to overcome such limitations. For their successful translation to clinical practice, further investigation regarding their toxicity profiles and bioavailability is required.

This study highlights the potential of the lipopeptide C_{12}OOWW-NH_2 for the treatment of FK and introduces a new *ex vivo* model for reproducible evaluation of the antimicrobial activity and toxicity profile of such antifungal therapeutics.

Methods:

Mass spectroscopy was used to determine the lipopetide identy following synthesis, while HPLC was used to confirm lipopeptide purity. Secondary structure analysis of the lipopeptide was conducted using circular dichroism. Anti-microbial activitiy, characterised by minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), was determined using an anti-microbial activity assay. Cell viability was assessed using various assays including, MTS cell viability, LIVE/DEAD staining® with fluorescent microscopy and haemolysis. The antimicrobial activity and cytotoxicity of the lipopeptide was examined *ex vivo* using a rat corneal model.

Results:

Analysis conducted by circular dichroism showed 2.99 % and 20.87 % α-helical and β-strand composition of the lipopeptide, respectively. Broad-spectrum activity was demonstrated against a range of fungi clinically relevant to infection, with MIC values of lipopeptide C_{12}OOWW-NH_2 ranging between 7.812 and 15.625 µg/mL and MFU values ranging between 15.625 and 31.25 µg/mL C_{12}OOWW-NH_2 led to a 90 % (1 log) reduction in log CFU within infected rat corneas *ex vivo*, versus the control. No toxicity was observed against human retinal cell line (ARPE-19 cells) and similarly no apparent red blood cell haemolysis occurred.

Conclusions:

This study demonstrates the promising ability of C_{12}OOWW-NH_2 to treat FK in a safe and efficacious manner, whilst also developing a novel *ex vivo* model that provides reproducible evaluation of such lipopeptides in the treatment of FK.

References

[1] F. Bongomin, S. Gago, R. O. Oladele, and D. W. Denning, “Global and multi-national prevalence of fungal diseases—estimate precision,” *J. Fungi*, vol. 3, no. 4, 2017, doi: 10.3390/jof3040057.