Assessing the impact of DEC-RVKR-CMK on *Cryptococcus neoformans* cells

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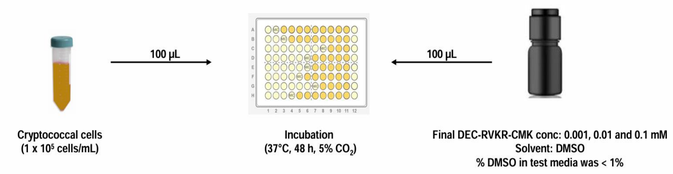
Introduction

Cryptococcus (C.) neoformans is a basidiomycetous yeast that rose from being an obscure fungus to an important fungal pathogen. Part of its success is attributed to its arsenal of virulence factors that allow it to subvert the immunological response in a susceptible host. This includes the production of proteases that Tucker and Casadevall (2002) reasoned could permeabilise the phagosomal membrane leading to internalised cells escaping.

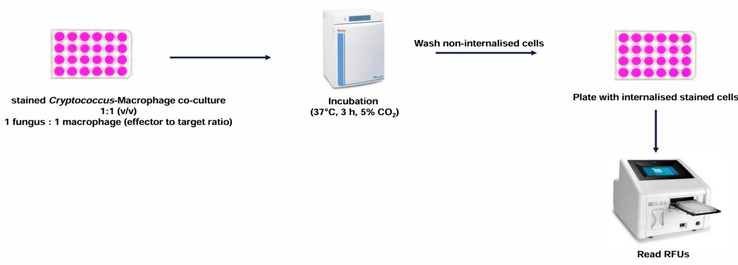
In this project, it was sought to assess if a protease inhibitor, decanoyl-RVKR-chloromethyl ketone (DEC-RVKR-CMK), could decrease cryptococcal growth and increase their susceptibility towards macrophage phagocytosis. This compound is routinely used to control unwanted proteolysis in model viral entry studies, including HIV entry studies. Interestingly, *C. neoformans* often manifests in advanced HIV disease.

Methods

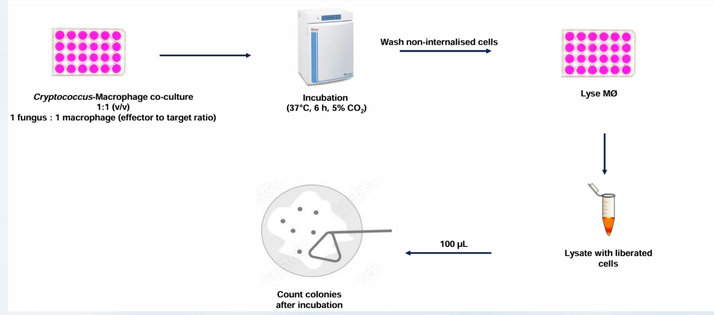
Effect of DEC-RVKR-CMK ON growth



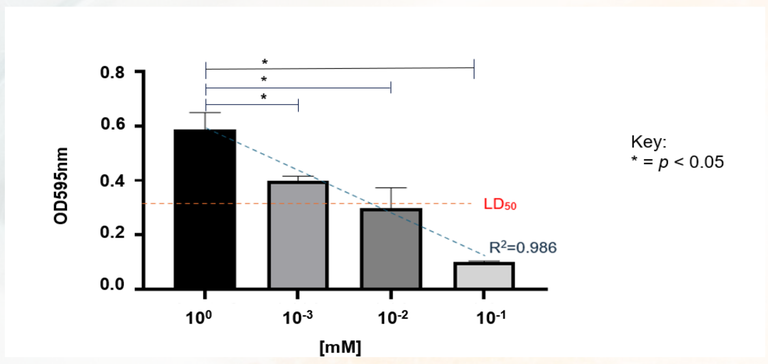
Effect of DEC-RVKR-CMK on Mϕ internalisation



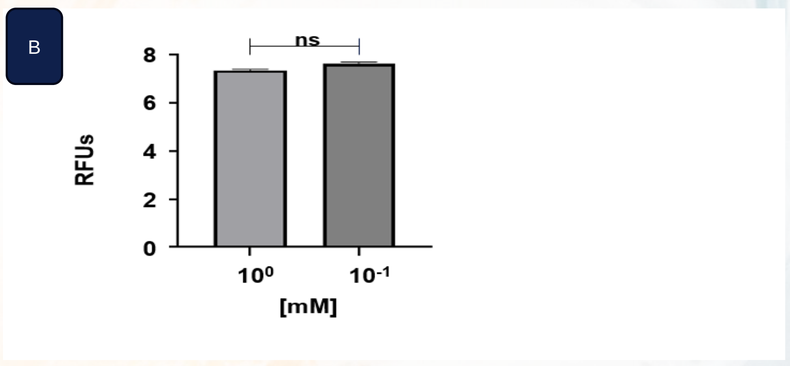
Effect of DEC-RVKR-CMK on Mϕ phagocytosis



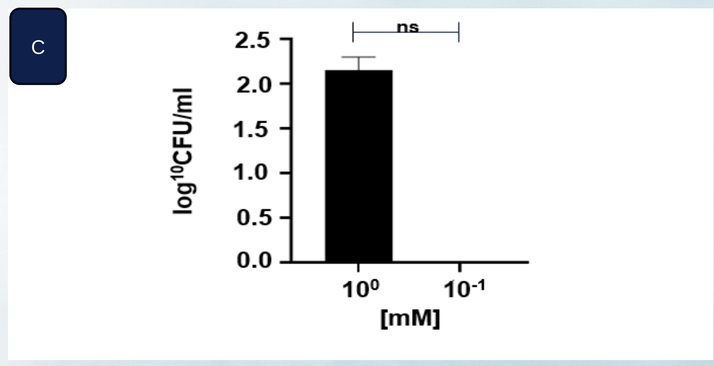
Results



(A) DEC-RVKR-CMK inhibited the growth of cryptococcal cells in a dose-dependent manner. The 0.1 mM was defined as the MIC, as it inhibited more than 70% of growth, and was used in the subsequent experiments



(B) There was no statistical difference in the efficiency of macrophages to uptake and internalise cryptococcal cells that were exposed to 0 mM and 0.1 mM of DEC-RVKR-CMK.



(C) Of the cryptococcal cells that were internalised, those exposed to 0.1 mM of DEC-RVKR-CMK were more susceptible to macrophage phagocytosis compared to those exposed to 0 mM.

Discussion and conclusion

- DEC-RVKR-CMK was repurposed as an anti-*Cryptococcus* compound and was shown to inhibit cellular growth and increase the cells' susceptibility to macrophage killing.
- While not investigated in the current study, the DEC-RVKR-CMK success may be attributed to its ability to inhibit proteases, which are essential for cellular growth and virulence.
- More to this point, proteases catalyse the maturation of laccases, which in turn are critical for the production of melanin, one of the principal microbial factors of *C. neoformans* essential for cellular growth and virulence.
- The clinical relevance of the study may lie in DEC-RVKR-CMK impairing the ability of cryptococcal cells to escape from macrophages, as cells often use vomocytosis to evade immunoprocessing.
- It is now prudent to investigate the molecular mechanism by which DEC-RVKR-CMK increases susceptibility to macrophage phagocytosis.