Repurposing morpholine and allylamine drugs as anti-*Acanthamoeba* agents

Logan Mackie[1], Leonor Maciel[2], Maria Cardoso[2], Craig W. Roberts[1]

1. Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, UK

2. Faculdade de Farmácia da Universidade do Porto, Universidade do Porto, R. Jorge de Viterbo Ferreira 228, 4050-313 Porto

Acanthamoeba are opportunistic pathogens that represent the source of 2% of the global corneal infections by causing *Acanthamoeba* Keratitis (AK). Alongside this, immunosuppressed individuals are vulnerable to granulomatous amoebic encephalitis (GAE), a rare central nervous system (CNS) infection with a 90% mortality rate. As an orphan disease, *de novo* drug development has not proven financially attractive, and much effort has focused on repurposing drugs. In this respect and not surprisingly considering the relative phylogenetic proximity of Amoebazoa to fungi, anti-fungals have been a fertile area for anti-*Acanthamoeba* infection and have shown some success. As sterol biosynthesis in humans and *Acanthamoeba* differ, disrupting this pathway presents a potentially exploitable mechanism with low host toxicity. Herein, we investigate anti-fungal drugs of the morpholine and allylamine class as potential anti-*Acanthamoeba* agents, utilising alamarBlue viability assays and microscopy. We further investigate the cysticidal effects of the most effective drug using microscopy, as cysts can persist within the host for years before becoming reactivated. The allylamine drugs terbinafine and butenafine significantly reduced trophozoite viability in a dose-dependent manner (IC50s 135ng/ml and 1125ng/ml respectively), whilst the morpholine drug Amorolfine is less efficacious (IC50 5491ng/ml) Washout studies highlight that upon removal of the drug and replacement of growth media, both high concentrations of terbinafine and butenafine, but not amorolfine were trophocidal. Further studies found that treating *Acanthamoeba* cysts with terbinafine reduced their viability. These data highlights the effectiveness of early-pathway squalene epoxidase inhibitors exemplified by terbinafine as potential anti-*Acanthamoeba* drugs. Further studies using combination therapies including squalene epoxidase inhibitors may enhance the action of current agents without the need for costly development of drugs for this orphan disease.