**Abstract**

**Title:**

Inhibition of fatty acid oxidation: a new treatment strategy for Primary Amoebic Meningoencephalitis?

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**Abstract text (4000 characters maximum, now 2801)**

Background

The free-living amoeba *Naegleria fowleri* causes Primary Amoebic Meningoencephalitis (PAM), a rapidly fatal disease of the central nervous system (CNS). This brain-eating parasite can enter the CNS by migrating from the nasal epithelium along the nasal nerve to the olfactory bulb. Symptoms include severe headache, fever and seizures, with deterioration into coma and eventually death. Current treatment consists of a wide range of antifungals and antibiotics. However, despite this extensive treatment, prognosis is extremely poor with a fatality rate of over 95% within 2 weeks of symptom onset. Previous experiments performed in our laboratory showed that *Naegleria gruberi*, a non-pathogenic close relative of *N. fowleri*, prefers fatty acids as a food source. This could provide a new treatment strategy against PAM.

Methods

*N. gruberi* ATCC 30224 was used to evaluate the effects of several fatty acid oxidation inhibitors in comparison to the current treatment. A high throughput *N. gruberi* growth monitoring assay using optical density was developed to evaluate inhibitory effect of compounds on growth. Compounds were tested on their own as well as in combinations. In addition to growth inhibition, the killing capacity of the compounds was assessed by analysis of regrowth after washing away the compounds.

Results

Several inhibitors of fatty acid oxidation restricted in vitro growth (Valproic acid (VPA), Orlistat (ORL), Thioridazine (TDZ)) and some also prevented regrowth (Perhexiline (PHX), Etomoxir (ETO)). Current treatment (Amphotericin B (AmB) and Miltefosine) was shown to be effective as well, which validates our high throughput assay. Additive effects were observed when VPA was combined with any of the other fatty acid oxidation inhibitors. Furthermore, killing was achieved when VPA, ETO or PHX was combined with AmB, while single used drugs did not show killing capacity.

Discussion

Repurposing drugs is the only way to obtain new treatment options for PAM, as the rapid fatal nature of the disease does not allow randomized controlled trials to be performed. Using an hypothesis-driven approach, we identified several currently used drugs that inhibit amoebal growth. Two of these (VPA and TDZ) showed effectivity at concentrations attainable in the human brain, in contrast with current therapy (AmB and MIL), which cannot reach therapeutical concentrations at the site of infection. Furthermore, VPA and TDZ showed an additive effect on growth inhibition when these drugs were combined. As the primary effect of VPA also relieves one of the symptoms of PAM, we believe this in vitro research can be rapidly applied in the clinic.