

JMM Correspondence

Primary amoebic meningoencephalitis: amoebicidal effects of clinically approved drugs against *Naegleria fowleri*

Naegleria fowleri is a protist pathogen known to produce fulminating primary amoebic meningoencephalitis (PAM) (Marciano-Cabral & Cabral, 2007; Visvesvara *et al.*, 2007). Although rare, PAM is a serious human disease with a fatality rate of >95%. It is generally associated with swimming in contaminated freshwater and possibly through nasal irrigation for cleansing (Marciano-Cabral & Cabral, 2007; Visvesvara *et al.*, 2007). PAM cases have recently been observed in Muslims who routinely perform ritual ablution, which involves repeated water exposure to the nostrils for cleansing (Siddiqui & Khan, 2011). These so-called 'brain-eating amoebae' invade the nervous system via the nose when contaminated water is deeply inhaled into the nose, and migrate to the brain tissue where severe haemorrhaging and inflammation are caused, resulting in widespread brain tissue destruction (Marciano-Cabral & Cabral, 2007; Visvesvara *et al.*, 2007). As well studied as this protist is, we still do not know of any effective chemotherapeutic interventions. The current treatment regimen involves a mixture of drugs ranging from antimicrobial compounds to experimental anti-cancer drugs (Marciano-Cabral & Cabral, 2007; Visvesvara *et al.*, 2007), to provide additive or synergistic effects, but even then the mortality remains very high (~98%) (Marciano-Cabral & Cabral, 2007; Visvesvara *et al.*, 2007). This is probably due to difficulties in early diagnosis, resulting in delayed initiation of effective chemotherapy, and/or poor penetration of antimicrobial compounds across the blood-brain barrier. With the devastating nature of this disease and the problems associated with its chemotherapy, the overall aim of the present study was to test anti-*N. fowleri* effects of clinically available drugs *in vitro*.

All chemicals were purchased from Sigma, unless otherwise stated. Among the various drugs tested, amlodipine, apomorphine and loperamide were purchased from

Sigma, procyclidine was purchased from Auden McKenzie Pharma, haloperidol was purchased from Searle Pharma, amiodarone was purchased from Sanofi-Aventis and digoxin was purchased from GlaxoSmithKline. The drugs tested in the present study and their known mode(s) of action are listed in Table 1. All drugs tested are available clinically and approved by the Food and Drug Administration and/or European Union drug regulatory authorities, whilst some are used to treat nervous system and cardiovascular disorders (Brunton *et al.*, 2011).

A clinical isolate of *N. fowleri*, sourced from cerebrospinal fluid of a patient who died recently of PAM, was grown in 10 ml Nelson's medium (Marciano-Cabral & Cabral, 2007; Visvesvara *et al.*, 2007). The isolate was maintained in culture for 1 month and its identity confirmed with PCR using *N. fowleri*-specific primers (Yousuf *et al.*, 2013) before testing in this study. The medium was refreshed 15–20 h prior to experiments. To determine the lytic effects of drugs on *N. fowleri*, amoebicidal assays were performed. Briefly, *N. fowleri* trophozoites were incubated with different concentrations of drugs (10–250 μM) in Page's amoeba saline (10^5 trophozoites ml^{-1} per well) in 24-well plates. Plates were incubated at 37 °C for 24 h. Following this incubation, lytic effects were determined by haemocytometry, whilst amoebae viability was determined by inoculating drug-treated amoebae in fresh growth medium (Nelson's medium). In some experiments, 0.1% trypan blue was added, and numbers of live (non-stained) and dead (stained) amoebae were counted. *N. fowleri* incubated with amoebic saline alone was used as controls. Data are representative of at least three experiments performed in duplicate and are expressed as mean \pm SE.

The amoebicidal effects of procyclidine, digoxin, amlodipine and haloperidol were

determined at micromolar concentrations. *N. fowleri* was treated with 200 μM of the different drugs, targeting vital receptors and biochemical pathways. The percentage of amoebae in controls was considered as 100% and the effects of drugs are expressed as relative change. The findings revealed that 200 μM digoxin showed significant lytic effects ($P < 0.01$ using a paired *t*-test; one-tail distribution) as observed by a >90% reduction in amoebae numbers (Fig. 1). Furthermore, no viable amoebae were observed post-treatment when inoculated in the growth medium for 48 h (data not shown). Even longer incubations did not allow the re-emergence of viable amoebae. Similarly, procyclidine showed potent amoebicidal effects (Fig. 1). Amlodipine showed a reduction in amoebae to $19 \pm 1.2\%$ compared with 100% for the controls. Haloperidol and apomorphine reduced the amoebae to 21.6 ± 4.8 and $33 \pm 0.4\%$, respectively, whilst amiodarone and loperamide showed reductions to 51.8 ± 9 and $73.8 \pm 1.6\%$, respectively.

Overall, of the seven drugs tested, two (digoxin and procyclidine) showed effective killing of *N. fowleri*, whilst amlodipine showed >80% amoebicidal effects. Amlodipine is a dihydropyridine calcium channel blocker used in the treatment of systemic hypertension and myocardial ischaemia (Brunton *et al.*, 2011; Baig *et al.*, 2013). Digoxin is a widely used positive inotropic drug that is given in congestive heart failure and supraventricular tachycardia that acts through inhibition of Na/K-ATPase (Brunton *et al.*, 2011; Baig *et al.*, 2013). Digoxin crosses both the blood-brain barrier and the placenta. Following intravenous administration to healthy volunteers, 50–70% of the digoxin dose is excreted unchanged in the urine, and only a small percentage (~16%) of the given dose is metabolized with a half-life of ~36 h. The end metabolites include 3- β -digoxigenin, 3-keto-digoxigenin, and their

Table 1. Clinically available drugs tested in the present study and their known mode(s) of action

Drug	Mode(s) of action
Amiodarone	Class III antiarrhythmic agent, shows β -blocker-like and potassium-channel-blocker-like actions Binding to the nuclear thyroid receptor
Amlodipine	Calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle Acts as a functional inhibitor of acid sphingomyelinase; sphingomyelin is involved in signal transduction and apoptosis (cell death)
Apomorphine	Non-selective (D) dopaminergic receptor agonist An antagonist at 5-hydroxytryptamine and α -adrenergic receptors
Digoxin	Binds to Na/K-transporting ATPase α 1 chain to inhibit its function at heart muscle cells and other tissues Inhibitor of cholesterol side-chain cleavage enzyme, mitochondrial
Haloperidol	Dopamine antagonist Muscarinic receptor antagonist Histamine antagonist
Loperamide	μ -Opioid receptor agonist Calmodulin binder
Procyclidine	Blocks the neurotransmitter acetylcholine in the central and the peripheral nervous system at muscarinic receptors

glucuronide and sulfate conjugates (Brunton *et al.*, 2011). Procyclidine is an anti-cholinergic agent that is widely used as an anti-parkinsonian agent because of its anti-muscarinic action (Brunton *et al.*, 2011; Baig *et al.*, 2013). Procyclidine primarily antagonizes muscarinic receptors M1, M2 and M4 (Brunton *et al.*, 2011; Baig *et al.*, 2013). Another agent, haloperidol, a drug of the same class as trifluoperazine, produced potent amoebicidal effects. Haloperidol acts primarily as a dopamine receptor blocker and has been used as an anti-psychotic drug. This is consistent with previous studies

which showed that chlorpromazine is effective against *Acanthamoeba castellanii* (reviewed in Baig *et al.*, 2013). Among the other drugs tested, apomorphine is a non-selective dopamine agonist that activates both D₁- and D₂-like receptors, with a preference for the latter subtype (Brunton *et al.*, 2011; Baig *et al.*, 2013). Amiodarone resembles thyroxin (a thyroid hormone) chemically and its binding to the nuclear thyroid receptor might contribute to its pharmacological properties.

In conclusion, to the best of our knowledge, we have shown for the first

time that a diverse group of drugs (some of which are used currently for central nervous system disorders) (Brunton *et al.*, 2011; Baig *et al.*, 2013) affecting cellular availability of calcium, sodium and potassium ions through G-protein-coupled receptors and metabotropic receptors showed anti-*N. fowleri* effects. Future studies will explore the molecular mechanisms to validate the mode of action of these drugs *in vitro* and *in vivo*. It is believed that the findings reported here will be of potential value in the rational development of therapeutic interventions

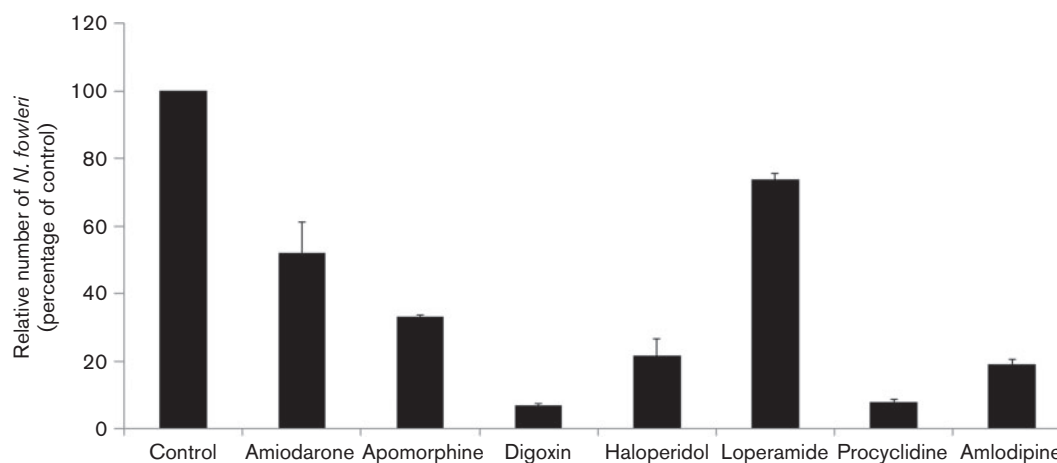


Fig. 1. Lytic effects of various drugs determined by haemocytometry. Briefly, *N. fowleri* (10^5 amoebae) were incubated with and without various drugs (200 μ M) at 37 °C for 24 h in Page's amoeba saline, followed by counting of amoebae. The percentage of amoebae in controls was considered as 100% and the effects of drugs are expressed as relative change. At 200 μ M, digoxin and procyclidine showed potent lytic effects. The results are representative of three independent experiments performed in duplicate. Data are presented as mean \pm SE.

and open up new fields of research against the devastating effects of PAM.

Acknowledgements

This work was partially supported by grants from The Aga Khan University. The authors declare no conflicts of interests.

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