

JMM Correspondence

Recommendations for the management of *Acanthamoeba* keratitis

Acanthamoeba is the causative agent of a painful, sight-threatening keratitis. The first case of *Acanthamoeba* keratitis was reported in 1972 in the USA and soon after, it was reported in the UK in 1974 (Nagington *et al.*, 1974). Ulcerative keratitis due to *Acanthamoeba* is often associated with the improper use of contact lenses, but it is also reported in non-contact lens wearers (Sharma *et al.*, 2000). At present, treatment involves hourly topical application of a mixture of anti-*Acanthamoeba* drugs including 0.02 % polyhexamethylene biguanide or chlorhexidine for up to a week. The hourly drops may be reduced after 48 h to alleviate the epithelial toxicity, but treatment continues for up to a year (Clarke *et al.*, 2012).

In a recent report by Agahan *et al.* (2009), it was demonstrated that three patients with *Acanthamoeba* keratitis were treated successfully without any anti-*Acanthamoeba* drugs. The patients had no history of contact lens wear and were admitted due to intolerable eye pain and blurring of vision. Ocular examination revealed corneal ulceration and stromal infiltrates. The patients were reportedly diagnosed with *Acanthamoeba* keratitis (Agahan *et al.*, 2009). All patients were treated for photophobia with anticholinergics (atropine). In ophthalmologic practice, atropine is commonly used for ciliary muscle paralysis to block accommodation and to prevent reflex constriction of pupillary muscle. For inflammation and pain, the patients were given topical diclofenac sodium (0.1 %) every hour for up to a week. The patients responded well to this combination. Based on this, it was suggested that the successful prognosis was due to suppression of the inflammation. Subsequently, the treatment was continued for up to 3 months, with one case receiving it for up to 10 months. As the patients were recovering well, no anti-*Acanthamoeba* agents were introduced. No corneal melt or rupture was observed and no

recurrence was seen for at least 12 months of the follow-up (Agahan *et al.*, 2009). In another case of *Acanthamoeba* keratitis, atropine eye drops added to conventional anti-*Acanthamoeba* agents with oral diclofenac and steroids restored the vision (Stapleton *et al.*, 2009).

The aim of this letter is to provide the scientific basis of the successful prognosis observed in these patients in the absence of anti-*Acanthamoeba* agents. Notably, recent studies have shown potent anti-*Acanthamoeba* effects of the anticholinergic agent procyclidine *in vitro* (Baig *et al.*, 2013). Procyclidine is generally given in less advanced forms of Parkinson's disease. It is believed to act by reducing the effects of acetylcholine (Sweeney, 1995; Olanow & Koller, 1998). Based on this, we hypothesize that the successful prognosis observed in the above *Acanthamoeba* keratitis cases were not merely due to regulation of the inflammatory process, but were most likely due to the anti-muscarinic effects of atropine, leading to killing of the *Acanthamoeba*. In support of this notion, we report here that atropine acts as an anti-*Acanthamoeba* agent. Briefly, amoebae (5×10^5) were incubated with 200 μg atropine ml^{-1} and cytotoxicity was determined by adding 0.1 % trypan blue and enumerating the number of live (non-stained) and dead (stained) *Acanthamoeba castellanii* using a haemocytometer. The counts from *A. castellanii* incubated with PBS alone were used as controls. The results revealed that atropine produced a kill rate greater than 55 ± 4.5 %. The data are representative of at least three independent experiments and expressed as mean \pm SE.

Among five muscarinic receptors targeted by atropine, we further determined the receptor subtype involved in atropine-mediated *A. castellanii* death. The use of dicyclomine, which selectively targets the M1 receptor, resulted in more than 75 % parasite killing within 8 h at a concentration of 90 μM . Agents targeting

the M1 receptor thus should prove highly effective in the treatment of this blinding infection.

Additionally, we propose amlodipine as a substitute anti-inflammatory agent (Kataoka *et al.*, 2004) for diclofenac sodium eye drops. The anti-inflammatory effects of amlodipine are probably mediated by the inhibition of monocyte chemokine protein-1, tumour growth factor-1, the Rho pathway and oxidative stress (Kataoka *et al.*, 2004). Moreover, our recent findings showed potent amoebicidal properties of amlodipine (Baig *et al.*, 2013), and thus its use is likely to lead to dual effects in the successful management of *Acanthamoeba* keratitis.

In conclusion, we suggest the use of the selective muscarinic antagonist dicyclomine rather than atropine, as well as topical amlodipine to substitute for diclofenac sodium in cases of *Acanthamoeba* keratitis. The proposed use of these drugs needs to be tested in clinical trials to prove their efficacy in amoebic keratitis.

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