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Applied Microbiology 2019: Impact of commonly used topical agents and anti-acanthamoeba drugs on *Acanthamoeba spp*. Viability- Anas Hamad-University of Wolverhampton

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Acanthamoeba is a genus of small, free-living amoebae common to most land and freshwater habitats. The body has a life cycle of feeding and replicating trophozoite which, in response to adverse conditions, can form a dormant cyst stage. Its *Acanthamoeba* spp. Opportunistic human pathogens cause lethal granulomatous encephalitis in the immunocompromised host and, more commonly, potentially blinding keratitis in both non-contact lens and Contact Lens (CL) wearers.

There are currently around 4.1 million CL wearers in the UK and established independent risk factors for developing *Acanthamoeba* Keratitis (AK) in CL wearers include exposure to tap water at home, swimming or bathing while wearing CL, poor lens hygiene and the use of rigid CLs in orthokeratology. In addition, previous outbreaks of AK in both the United Kingdom and the United States were due to effectiveness issues with some CL disinfection systems. Given the sight-threatening risk with AK, it accounts for less than 5 percent of all CL-related microbial keratitis in most episodes. The published incidence rates for CL users are 1 to 2 per million in the United States and 17 to 20 per million in the United Kingdom. A recent research in a tertiary hospital in the United Kingdom showed an incidence rate of only 2.3 per cent for *Acanthamoeba* in more than 1500 cases of keratitis over a 12-year period. Due to the small number of patients with AK, many are diagnosed late because they were initially misdiagnosed and treated for bacterial or other forms of keratitis, such as fungal and herpes simplex keratitis. Early diagnosis of AK has a substantial effect on prognosis, and patients are more likely to have worse visual outcomes, longer period of treatment, corneal perforation, and a need for penetrating keratoplasty. Traditional medical treatment for AK is unlicensed and requires topical administration of biguanide, i.e. 0.02 per cent PolyHexaMethylene Biguanide (PHMB) or 0.02 per cent chlorhexidine, either as monotherapy or in conjunction with 0.1 per cent propamidine or 0.1 per cent hexamidine. It has been reported that PHMB and chlorhexidine are the most effective against both trophozoites and *Acanthamoeba* cysts.

Methods

Amoebicidal and cysticidal assays were performed against both the trophozoites and cysts of *Acanthamoeba castellanii* (ATCC 50370) and *Acanthamoeba polyphagae* (ATCC 30461). Testing agents included topical ophtalmic preparations of specific anesthetics, antivirals, antibiotics and biocides. Organisms were exposed to serial two-fold dilution of the test compounds in the microtiter plate wells to examine the effect on *Acanthamoeba* spp. In addition, the toxicity of each test compound was assessed against the mammalian cell line.

Results

The inhibitory range against *Acanthamoeba* trophozoites for proxymetacaine, tetracaine and oxybuprocaine anesthetics was 9.75 to 39 µg / ml, while lidocaine provided no growth inhibition until the 312-to 625-µg / ml range for both organisms. In the trophozoite amoebicidal tests, proxymetacaine, tetracaine, and oxybuprocaine were amoebicidal in the 19.5 to 250 µg / ml range, while the amoebicidal activity against A was lidocaine. Polyphage and *A. Castellani* was 312 and 1250 µg / ml, respectively. For cyst assays, proxy-metacaine, tetracaine, and oxybuprocaine were cysticidal in the 39-to 250-µg / ml range. For lidocaine, anti-A cysticidal operation. Polyphage and *A. Castellani* were 1.25 and 10 mg / ml respectively. In the mammalian cell line toxicity assay, proxymetacaine, tetracaine, and oxybuprocaine were cytotoxic in the 39-to 156-µg / ml range, while lidocaine was not cytotoxic before 5 mg / ml. Lidocaine Minims contains sodium fluorescein that has been checked independently for regulation and found to be non-toxic at a concentration of 2 per cent. Proxymetacaine, oxybuprocaine, and tetracaine in particular, were all toxic to *Acanthamoeba* spp. trophozoites and cysts but lidocaine was well tolerated.

The presence of benzalkonium chloride (BAC) preservatives in levofloxacin has resulted in a high degree of toxicity to trophozoites and cysts. The involvement of BAC in the propamidine drops was responsible for the operation against *Acanthamoeba* spp. Hexamidine drops without BAC showed strong activity against trophozoites, and the biguanides polyhexamethylene biguanide, chlorhexidine, alexidine, and octenidine all displayed outstanding activity against both types of trophozoites and cysts.

Conclusions

Based on the antibiotic technique used, empiric treatment with fluoroquinolones of third or fourth generation due to their wide-spectrum antimicrobial activity is frequently used as an initial therapy for the treatment of microbial keratitis. We observed that the pure levofloxacin product and the preservative-free preparation of moxifloxacin (Moxeza) did not have a

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significant antimicrobial effect on the viability of *Acanthamoeba*, while the modified levofloxacin (Oftaquix), which is modified with BAC, had a much higher antimicrobial activity on both species of *Acanthamoeba*. This indicates that it is the BAC that causes the antimicrobial effect observed in preserved levofloxacin rather than the drug itself. Thompson and coworkers did not notice any adverse effects on *Acanthamoeba* PCR amplification with gatifloxacin or moxifloxacin. The gatifloxacin used in their analysis (Zymar; Allergan, Irvine, CA) was maintained with BAC, while moxifloxacin was self-preserved. While BAC has not been tested on its own, the limited inhibitory effect of both antibiotics suggests that the impact of BAC on PCR in the detection of *Acanthamoeba* DNA may be lower compared to the amoebicidal and cysticidal assay methods used in this report.

The antiamoebic effects of BAC, povidone iodine and tetracaine are higher than current diamidines and slightly lower than those of the biguanides used in the treatment of *Acanthamoeba* keratitis. In conclusion, the present research indicates that the use of proxymetacaine, oxybuprocaine and tetracaine to reduce pain; ophtalmic preparations containing preservatives such as BAC; and the use of povidone iodine prior to sampling may affect the viability of *Acanthamoeba* in vivo, resulting in reduced crop yield and inhibition of PCR amplification.

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