LETTERS TO THE JOURNAL

Sir,

Drug Therapy in a Murine Model of *Acanthamoeba* Keratitis

Infection with *Acanthamoeba* has become an important cause of keratitis, primarily amongst soft contact lens wearers. The clinical picture is usually of a chronic indolent keratitis characterised by severe pain, formation of a pericentral ring infiltrate with a granular stromal opacity and recurrent epithelial breakdown leading eventually to corneal thinning and perforation, although earlier stages of infection are being increasingly recognised. The most appropriate therapy for *Acanthamoeba* keratitis remains uncertain, but a large number of antimicrobial drugs have been shown to have high *in vitro* activity against *Acanthamoeba* and various combinations have proved effective clinically. Recently Elder *et al.*[1] reported a poor correlation between the clinical outcomes of 23 culture-proven cases of *Acanthamoeba* keratitis and the results of *in vitro* sensitivity testing.

We undertook a study to assess the feasibility of using a rat model of *Acanthamoeba* keratitis described by Larkin and Easty[2] to determine the efficacy of different medical treatments *in vivo*. Briefly, the central corneal stroma of 60 male rats was inoculated with an axenic cyst suspension of *Acanthamoeba polyphagia*, and the resultant keratitis graded after 3 days by slit lamp examination (Table I). The animals were then treated in a masked fashion with either propamidine isethionate 1%, polyhexamethylene biguanide 0.02% (PHMB) or vehicle instilled every 6 hours for 14 days. Further clinical grading of keratitis was done on days 5, 7, 10, 13 and 16, after which the animals were killed and the corneas homogenised and cultured.

There was no significant difference in the mean grade of keratitis between the three groups prior to commencing, during or at the completion of treatment, using Fisher's test of variance (Table II). There was also no significant difference in the number of culture-positive corneas between the treatment groups using the chi-squared test with Yates' correction (Table III). It was thus not possible to demonstrate a beneficial effect of either agent as used in the rat model.

It is worth noting that the number of culture-positive corneas in the group treated with the vehicle alone was unexpectedly low. The rat cornea has proved relatively resistant to *Acanthamoeba* infection, and Neiderkorn *et al.*[3] have shown failure of *Acanthamoeba* trophozoites to bind to the epithelium of rat corneas when cultured *in vitro*, while they bound readily to the epithelium of human, pig and Chinese hamster corneas. Successful animal models have been established in Chinese hamsters and micropigs[4,5] in which the cornea was abraded and a parasite-laden contact lens worn for up to 7 days. Although these models seem more relevant to human keratitis as it is now recognised than the rat model we used, they have also resulted in self limited disease with eventual resolution of signs of active corneal inflammation and inability to culture *Acanthamoeba* from corneal specimens in the late stages.

Successful medical cure of *Acanthamoeba* keratitis has usually required prolonged intensive treatment with multiple agents as first described by Wright *et al.*[6] using a combination of dibromopropamidine, propamidine isethionate and neomycin. Larkin *et al.*[7]

Table I. Slit lamp clinical grading of *Acanthamoeba* keratitis

Grade 3	Corneal opacity visible on retroillumination obscuring iris detail
Grade 2	Corneal opacity visible on retroillumination but not sufficient to obscure iris detail
Grade 1	Corneal opacity visible only by oblique slit beam
Grade 0	No corneal opacity

Table II. Mean clinical grade of keratitis (and standard deviation) in different treatment groups according to number of days of treatment

Day	Propamidine	PHMB	Vehicle
0	2.05 (0.68)	2.20 (0.59)	2.00 (0.59)
2	1.90 (0.30)	1.95 (0.51)	1.75 (0.44)
5	1.60 (0.50)	1.80 (0.41)	1.65 (0.49)
8	1.50 (0.51)	1.65 (0.49)	1.55 (0.51)
11	1.45 (0.5)	1.70 (0.47)	1.60 (0.50)
14	1.35 (0.49)	1.30 (0.47)	1.35 (0.58)

PHMB, polyhexamethylene biguanide.

Table III. Corneal culture results according to treatment group

	Propamidine	PHMB	Vehicle
Culture-positive	5	8	9
Culture-negative	15	12	11

PHMB, polyhexamethylene biguanide.

were the first to describe the use of PHMB in the management of *Acanthamoeba* keratitis, and in one of their cases with early disease an apparent cure was effected after only 1 week of intensive treatment with this drug alone.

In our study single agents were used at an intensity and duration of treatment well below that in current clinical use, and drug levels reached in the cornea may not have been sufficiently high to kill trophozoites and cysts. It seems likely that if a beneficial effect of drug therapy in animal models is to be demonstrated intensive treatment with multiple agents for prolonged periods will be required.

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Sir,

Management of Superior Limbic Keratoconjunctivitis with Botulinum Toxin

Theodore's superior limbic keratoconjunctivitis is a disease which was first described in 1963. It is characterised by keratinisation and cellular infiltration of the superior bulbar conjunctiva, together with cellular infiltration of the upper palpebral conjunctiva. The disease is painful and the pain develops as the day progresses, usually reaching its maximum in a working individual in the late afternoon. The disease seldom interferes with sleep and is usually at its best on awakening.

The disease is difficult to treat successfully. Theodore reported treating the upper palpebral conjunctivae with silver nitrate 0.5–1.0% solution. This, in my experience, exacerbates the pain for 2 or 3 days and is followed by some relief which is never prolonged. I have never found the administration of drops to be of any value.

Some invasive treatments have been suggested, among them resection of the superior limbic and bulbar conjunctiva. Donshik *et al.*[1] claimed that all 4 of their patients had immediate and continued relief after this operation. Passons and Wood[2] stated that 8 of 10 of their patients who underwent this operation were either asymptomatic or much improved. Yet Darrell[3] found that 2 cases of superior limbic keratoconjunctivitis of 16 years' duration, in identical twins, never permanently responded to bilateral resection of the superior bulbar conjunctiva.

My experience with superior bulbar conjunctival resection has been mixed. I have performed the operation on 9 patients. I asked my patients to score the improvement in their symptoms by pointing to a scale marked from 0 to 10. Four failed to get relief, with scores of 0. Two had moderate relief, with scores of from 4 to 7. One of these achieved this score after having failed with botulinum toxin injections. Three had marked relief: 1 of these with a score of 8 and lost to long-term follow-up, and two had scores of 10, remaining so for 4 years.

Wright[4] proposed a mechanical cause for the condition and Ostler[5] postulated an upper lid in tight apposition to the superior bulbar conjunctiva, together with a lax superior bulbar conjunctiva, as the cause.

Accordingly I assessed the effect of botulinum toxin injections to the orbicularis muscle in patients with superior limbic keratoconjunctivitis. Generally I started with half the recommended dose given for essential blepharospasm. If there was no obvious blepharospasm associated with the superior limbic keratoconjunctivitis I tended to confine my injections to the superior orbital portions of the orbicularis muscles. Some of my patients had obvious blepharospasm and in these cases I injected the inferior orbital portions of the orbicularis as well.

The botulinum toxin used was Dysport (Speywood Pharmaceuticals, Maidenhead, UK). This is dispensed in a 500 unit vial to which is added 2.5 ml saline for injection. The injection sites are illustrated in Fig. 1. The doses indicated are full doses. (It must be emphasised that Botox, from Allergan Pharmaceuticals, Irvine, California, measured in units, is approximately three times more powerful than Dysport.)

Again I scored the success on a scale of 0 to 10. I