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Acanthamoeba keratitis successfully treated medically

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SUMMARY The first medical cure of a corneal infection due to an *Acanthamoeba* species is reported. The 44-year-old patient developed a suppurative keratitis associated with an epithelial defect, hypopyon, and secondary glaucoma. Acanthamoeba was confirmed as the causative agent four months after presentation when positive cultures were obtained from the cornea and from the conjunctiva. Sensitivity studies of the isolated organism were performed, and the infection was successfully controlled by treatment with a combination of dibromopropamidine and propamidine isethionate ointment and drops and neomycin drops. Keratoplasty was performed 22 months after onset, and no viable acanthamoebae were present in the resected tissue, though possible cyst remnants were identified by immunofluorescent techniques.

Infection of the eye with *Acanthamoeba* species has been reported many times[1-7] and has been associated with a fatal meningoencephalitis.[2] These infections are difficult to recognise and treat,[8] and acanthamoeba is usually considered only after failure of conventional treatment for more common causes of suppurative keratitis. No medical cure has yet been reported, and the outcome of all cases has been very poor, with excisional keratoplasty as the most successful diagnostic and therapeutic measure.

Case report

CLINICAL PRESENTATION

The patient was a 44-year-old meat porter, physically very fit and a marathon runner. In April 1979 he was splashed in the right eye with blood from a condemned carcass of meat but did not suffer any direct trauma to the eye. Within a few days the eye became red and sore and a variety of topical medicaments were given by the patient's general practitioner and local ophthalmologist including mydriatics, aureomycin, gentamicin, and neomycin with corticosteroid drops. Because of continuing deterioration the patient was referred to Moorfields Eye Hospital, where in June the findings were of a suppurative keratitis, hypopyon, and secondary glaucoma without any distinctive features. Corneal and conjunctival cultures and smears showed no organisms and no growth in any media but only pus cells and debris. A slow improvement in the signs followed subconjunctival and topical gentamicin, mydriatics, and topical dilute steroids with acetazolamide to control the intraocular pressure. The improvement was not maintained, and on several occasions the hypopyon increased for no obvious reason and then regressed again on continued treatment. This relapsing course continued for several months and in September the corneal epithelium broke down, with increasing stromal suppuration and hypopyon, producing an appearance similar to that seen in a patient subsequently shown to have acanthamoeba infections. Smears continued to show only pus cells and debris with no organisms. But special cultures for acanthamoeba from both cornea and conjunctiva grew *Acanthamoeba* sp. identified morphologically as *A. polyphaga* and continued to be positive for some days (Table 1). Nasal and pharyngeal cultures for acanthamoeba were also taken frequently and were all negative, as were cultures from the unaffected left eye.

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CULTURE TECHNIQUE

Petri dish plates were prepared with 1·5% Oxoid L28 agar, allowed to set, and left overnight at room temperature. *Aerobacter (Klebsiella) aerogenes* was grown in trypticase-soy broth or on nutrient agar, and the bacteria were washed by centrifugation three

times in phosphate-buffered saline (PBS). The bacterial pellet was resuspended to give a milky suspension in distilled water and stored (up to three months) at 4°C. Two to three drops were spread on the agar plates, allowed to absorb at 37°C, and the patient's samples were inoculated directly on to the surface of the plates. They were incubated at 37°C in a moist atmosphere for up to three days, and were examined for growth of amoebae by an inverted microscope. Further identification of the amoebae was by examination of trophozoites and cysts in suspension in Page's saline[9] under normal light and phase-contrast microscopy. On the basis of the presence of acanthopodia in the slowly moving trophozoite, and a polygonal double walled cyst with characteristic ostioles, the organisms were assigned to the genus *Acanthamoeba*.

TREATMENT

On the basis of previous sensitivity results from cases of acanthamoeba infection at Moorfields (discussed later) treatment was begun with drops of propamidine isethionate 0·1% hourly by day and night with dibromopropamidine ointment 0·15% four-hourly. After nine days of treatment the eye became much redder with rapidly worsening lid swelling despite improvement in corneal signs. These changes were attributed to toxicity, and since the sensitivity results for the organism cultured (Table 2) were now available treatment was changed to neomycin drops four-hourly day and night. By October the epithelium had healed, and four-hourly prednisolone drops were added, with steady improvement in the corneal inflammatory signs. The treatment was slowly reduced until the patient was using only neomycin drops four times daily. These were continued for one year, at which time limbal follicles developed with some increased palpebral conjunctival hyperaemia and cellularity but no sign of any skin irritation. All topical therapy was stopped and penetrating keratoplasty proposed, but not performed for a further four months at the patient's request for social reasons. During this period all the limbal and conjunctival signs disappeared, and no topical therapy was given.

An 8 mm penetrating keratoplasty was carried out 22 months after initial presentation. The postoperative treatment included propamidine isethionate drops four times daily in addition to the usual topical steroid and antibiotics employed. The propamidine drops were continued four times daily for two months without any evidence of adverse effect on the corneal graft or the remainder of the eye. After cessation of therapy the graft remained clear for nine months, when a rejection episode developed but was easily controlled with topical steroids. The graft remains clear at present and vision is 6/24, being reduced by lens opacities. The intraocular pressure is controlled with timolol maleate 0·25% drops twice daily.

Table 1 *Culture results*

	Right eye	
	Conjunctiva	*Cornea*
17 Sept.	+	+
20 Sept.	–	+
24 Sept.	–	–
27 Sept	–	–
30 Sept.	–	+
4 Oct.	–	–
8 Oct.	–	–
13 Oct.	–	–
15 Oct.	–	–
19 Oct.	–	
23 Oct.	–	
28 Oct.	–	

Nasal and nasopharyngeal cultures always negative

Histological examination of the excised corneal disc showed no special changes and no signs of acanthamoeba on light or electron microscope examination. By immunofluorescent techniques against acanthamoeba specific antigens it was possible to see small curved segments which took up the stain and which probably represented portions of broken up cyst wall.

SEROLOGY

Serum was taken at the time of positive corneal and conjunctival cultures and on four occasions during the following month. A low level of antibody was present with a slight rise, but the titres were not significantly raised above levels found in 'normal' sera (Table 3). Antibody could not be detected in serum or aqueous humour at the time of keratoplasty.

Table 2 *Relative sensitivities*

	Minimal inhibitory concentrations (μg per ml)	
	IT	*AmC*
Myxin	<1·2	2·4
Paromomycin	4·9	4·9
Ziram	4·9	9·8
Bayer 1703	>39·0	>39·0
Azalomycin	9·8	>19·5
Venturicidin	>312·0	>312·0
Neomycin sulphate	7·5	39·0
DS 9073	5·0	9·8
Propamidine	<1·25	<1·25
Dibromopropamidine	<1·25	2·5

IT=inhibition of trophozoite.
AmC=Amoebicidal (subculture negative).

Table 3 *Serology against acanthamoeba (Ryan strain)*

20 Sept.	1/16	13 Oct.	1/32
27 Sept.	1/16	19 Oct.	1/32
4 Oct.	1/16		

Discussion

This case differs from others reported in that it was not associated with direct ocular trauma or previous corneal ulceration, acanthamoebae were recovered directly from the cornea and the conjunctival sac, and the infection was subsequently controlled by topical therapy alone.

Although acanthamoeba infections of the eye have certain characteristic features, such as a slow relapsing course with development of ring abscess, recurrent epithelial breakdown, hypopyon which waxes and wanes, and secondary glaucoma, diagnosis is difficult and often long delayed. This is due in part to the need for special culture plates of plain agar preseeded with aerobacter to encourage growth of acanthamoeba and also because the organism cannot be identified on the usual corneal scrapings and smears which are taken and air dried to fix cells and bacteria. Such air drying causes rupture of these delicate organisms, leaving no distinctive residuum, but preserves cysts where present. Spray fixative such as is used for cervical cytology and other techniques are being tried to obtain better smear preparations, which should permit earlier diagnosis. Meanwhile the studies of sensitivity of the *Acanthamoeba* species identified from this case and others seen and treated at Moorfields Eye Hospital confirm that propamidine and dibromopropamidine isethionate are the most useful therapeutic agents in addition to neomycin, which has previously been suggested.

Drug sensitivity testing in vitro

The test was carried out as follows. A suspension of 100 000 to 250 000 trophozoites and cysts of acanthamoeba per ml was prepared in PBS. Washed live *Aerobacter aerogens* organisms grown in broth or on nutrient agar were suspended to an optical density of 6·0 at 650 nm in PBS. Drugs were prepared at a concentration 20 times that desired, also in PBS. 0·1 ml of amoebic and bacterial suspensions was added to 1·7 ml of PBS in sterile, screw-capped 10 ml test tubes, and 0·1 ml of drug solution was added. In control tubes 0·1 ml of PBS was added instead of drug solution. The test was carried out in duplicate. The tubes were incubated at 37°C for 48 hours. Five 0·02 ml samples were examined from mixed suspension from each tube to detect motile amoebae with typical acanthopodia. Where no apparently viable amoebae were found, the medium was centrifuged at 700 *g* for five minutes, and the sediment was inoculated on to non-nutrient agar plates coated with *A. aerogenes* and incubated further for 72 hours to detect any viable cysts or trophozoites.

Each experiment therefore gave two results, a minimum inhibitory concentration (MIC) for inhibition of trophozoite growth (MIC.I) and MIC for killing of trophozoites and cysts (MIC.AmC). Tests were initially carried out on an isolate of acanthamoeba from a corneal ulcer which was undergoing treatment at Moorfields Eye Hospital.[1] Subsequent less extensive tests with the most effective drugs were carried out on new isolates as they were obtained.[10] Table 4 lists the results of the original wide-ranging drug screen.

Propamidine and dibromopropamidine

Propamidine and dibromopropamidine isethionate have long been available as ocular therapeutic substances as drops and ointment (Brolene, Otamidyl, May and Baker antiseptic cream) and have been used in the treatment of blepharitis and also superficial skin infection such as that complicating burns. The compounds show antibacterial and antifungal properties in addition to the amoebicidal effect reported here. Dibromopropamidine isethionate is soluble 1 in 2 in water, and a 5% solution has pH 5·7. The commercially available preparation is a 0·15% cream (Brolene, May and Baker). It is reported to be active against pyogenic cocci, *Staphylococcus aureus*, Gram-negative organisms including *Escherichia coli*, *Proteus vulgaris*, and some strains of *Pseudomonas*. Propamidine isethionate is similar but less soluble in water, 1 in 5, and is commercially available as a 0·1% solution (Brolene eye drops). It shows a reduced spectrum of activity, being active against staphylococci and *Str. pyogenes* but inactive against *Pseudomonas*, *Proteus*, and *E. coli*.

The drops and ointment produced no serious adverse effects, but our experience of local irritation accords with the reported superficial necrosis of granulation tissue seen in some patients after topical application to wounds for more than 10 days. The initial treatment with Brolene was probably overintensive, but subsequent therapy with the drops four times daily was well tolerated for two months, suggesting that any initial intensive treatment period should not be for more than one week and thereafter treatment four times daily or less should suffice. The optimum treatment regimen remains to be determined, but propamidine and dibromopropamidine seem to offer the first effective medical treatment of this challenging group of ocular infections.

Table 4 *Drug sensitivity of acanthamoba*

Drug type	*Chemical group*	*Name*	*I*	*AC*	*MIC.I (μg/ml)*	*MIC.AmC (μg/ml)*
Trypanocide anti-Leishmania	Diamidines	Berenil diaceturate	+	+	<5	<5
		Stilbamidine isethionate	+	+	<5	<5
		20H stilbamidine isethionate	+	+	<5	<5
		Pentamidine isethionate	+	+	<5	<5
	Antimonial	Sodium stibogluconate	−	−	>156	>156
Antibiotic	Macrotetralide	Monensin sodium	−	−	>125	>125
	Macrolide	Venturicidin	+	+	2.5	156
	Polyene Macrolide	Candicidin	+	−	<25	>25
		Amphotericin B	−	−	>125	>125
		Azalomycin	+	+	9·8	9·8
	Polypeptide	Polymyxin B sulphate	+	+	39	313
		Gramicidin	+		313	
	Aminoglycoside	Paromomycin sulphate	+	+	9·8	9·8
		Neomycin sulphate	+	+	19·5	19·5
		Gentamicin sulphate	−		>156	
Fungicide		Miconazole	+	−	67·5	>250
		M & B 938	+	+	<5	<5
		Clotrimazole	−	−	>125	>125
	Bayer 1703	Clotrimazole analogue	+	−	<5	>156
		5-Fluorocytosine	+	−	156	>156
Antiviral		3-Fluorothymidine	−	−	>125	>125
Fungicide and antibacterial	8-Quinolinol	Steroxin	+	−	78	>156
		8-Hydroxyquinoline	+	+	9·8	78
Antianaerobe	5-Nitro imidazole	Metronidazole	−	−	>156	>156
Anthelmintic		Hetolin	+	−	39	>156
		Levamisole	+	−	<125	>125
		Diethylcarbamazine citrate	−	−	>156	>156
		Thiabendazole	−		>156	
Antiamoebic	Dichloroacetamide	Mebinol complex	+	+	55	438
Antimalarial Schizontocide		Mepacrine HCl	+	+	39	39
		Chloroquine diphosphate	−	−	>156	>156
PAB or folic analogues	Sulphonamide	Sulphamethoxazole	−	−	>156	>156
	Triazine	Trimethoprim lactate	−		>156	
Miscellaneous		Myxin	+	+	9·8	9·8
		Ziram	+	+	<5	9·8
		Fuberidazole	+	+	39	156
		Carnidazole	−	−	>156	>156

I=inhibition of trophozoite growth. AmC=amoebicidal (i.e., subculture negative). MIC=minimum inhibitory concentration.
+=Drug effective. −=No effect.

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