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Acanthamoeba Keratitis—Resistance to Medical Therapy

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Summary

Successful medical therapy of Acanthamoeba keratitis has been reported with combination therapy; topical Brolene and neomycin. Resistance has not so far been identified as a problem, but was the basis for recurrent disease observed in a patient with bilateral infection. Eradication of amoebae was finally achieved following prolonged topical therapy and two corneal grafts in each eye. Topical anti-amoebic therapy with paromomycin, benzethonium chloride, clotrimazole and R11/29 (a phenanthridinium compound), was continued for three months post-operatively. No further recurrences occurred during 14 months' follow-up. Drug sensitivities were performed for three isolates of *Acanthamoeba sp* (group II) which demonstrated the development of resistance to Brolene and arsenic. In addition, the resistant isolates were temperature-sensitive mutants which would not grow at temperatures above 30°C. This could explain 'culture-negative' results in some cases of clinical recurrence when incubation of laboratory samples had only been performed at 37°C.

Medical therapy of Acanthamoeba keratitis is only successful if commenced early when it can achieve eradication of the protozoa. The first medical cure, using combined topical neomycin and Brolene (dibromopropamidine and propamidine) was reported by Wright *et al,* 1985.[1] *In vitro* sensitivity testing has indicated a variety of drugs to be cysticidal, but with a wide range of minimum amoebicidal concentrations. Combination therapy has been widely adopted using propamidine, aminoglycosides, polypeptides and imidazoles; no clinical cure has resulted from single drug therapy. Published studies of acanthamoeba sensitivities to drugs have used stored clinical isolates of different amoebic strains and most strains are sensitive to the aminoglycosides and diamidines. Drug resistance has not yet been identified as a major clinical problem although *in vitro* sensitivity testing is not necessarily reflected by clinical response. Resistance should be considered, however, as an explanation for an arrested therapeutic response or for clinical recurrence.

Case Report

A healthy 29 year old male presented (21.7.87) with a three week history of bilateral keratitis. He had worn daily wear soft contact lenses while swimming in a chlorinated pool. Acanthamoebae were cultured at 37°C from the corneal scrapes. Treatment was started with hourly topical neomycin (0.5%) and Brolene (propamidine isethionate 0.1%) and the keratitis resolved steadily. The neomycin was stopped after one month due to epithelial toxicity and Brolene (6 hourly) was continued alone. The clinical course of each eye is reported separately.

Recurrence in the right eye followed withdrawal of neomycin. Acanthamoebae were isolated at 37°C from cultures of a corneal

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scrape of the focal infiltrate. Topical neomycin (6 hourly) did not achieve improvement and acriflavine 0.05% (3 hourly) was substituted for neomycin, but corneal infiltration increased in an arcuate fashion. A topical arsenical compound (R6/56, 0.5 g/l, courtesy of the late Dr E Friedheim, Rockefeller University, New York) was substituted and hourly therapy resulted in initial clinical improvement, but the response was not maintained.

The arsenical compound R6/56 is derived from Atoxyl with a substituted melaminyl radical. It is trypanocidal and filaricidal and has been found experimentally to be acanthamoebicidal at 10 μg/ml and inhibitory at 5 μg/ml. A topical preparation, 1 mg/ml, was found to be well tolerated by laboratory animals. This concentration was therefore used clinically.

As the keratitis was uncontrolled, a corneal biopsy was performed. Acanthamoebae were not grown in culture at 37°C (5 days), but did grow at 25°C after 10 days. This temperature-sensitive mutant did not respond to hourly topical imidazoles and polypeptides (1% miconazole and 0.25% polymyxin B). Further corneal scrapes were done, (24.3.88) for drug sensitivity testing. The isolates remained temperature-sensitive and were resistant to Brolene and arsenic.

The development of an intumescent cataract in the right eye necessitated an 8.5 mm penetrating keratoplasty (PK), extracapsular lens removal and implantation of a posterior chamber PMMA lens. Post-operative recurrence supervened and threatened wound stability, despite therapy with two hourly 2.5% paromomycin and 0.1% benzethonium chloride. A further 8.5 mm PK was therefore performed, (4.9.88) for uncontrolled recurrence. Paromomycin and benzethonium chloride were continued (2 hourly) post-operatively, but recurrence (20.10.88) required additional two hourly topical clotrimazole (1%) and 1% R11/29 (a polycyclic phenanthridinium compound, courtesy of the late Dr E Friedheim, Rockefeller University, New York). No further amoebae were isolated after this polytherapy was initiated and the keratitis settled although the graft failed.

Recurrent corneal disease occurred in the left eye, (8.12.87) after early withdrawal of two hourly neomycin therapy. Restarting 0.5% neomycin did not achieve improvement and left corneal biopsy was performed, (18.1.88). The arsenical compound R6/56 was given two hourly instead, (5 μg/ml) but initial improvement was not maintained. A further biopsy was done (24.3.88) which demonstrated a temperature-sensitive isolate and drug sensitivities of the mutant were performed. Hourly polymyxin B (0.25%) did not achieve improvement and an 8 mm PK was performed for uncontrolled disease and incipient perforation. The resistant mutant was again isolated. Infection recurred in the graft which perforated, (23.6.88), requiring an emergency PK. Topical 2.5% paromomycin and 0.1% benzethonium chloride were given post-operatively. Disease was controlled after the second keratoplasty. Anti-amoebic therapy was discontinued (10.1.89) (about 3 months post-operatively) and no recurrence has occurred in 16 months. The left graft remains clear with 6/12 unaided vision.

Methods

Acanthamoebae were cultured from corneal specimens by inoculating them centrally onto non-nutrient agar plates which were then seeded with *Escherichia coli* (washed in phosphate-buffered saline, PBS). Plates were incubated at 37°C for three days; if there was no growth of acanthamoebae, plates were then incubated at 25°C for 10 days. Acanthamoebae were identified from the morphological appearance of the trophozoites, characterised by the intermittent presence of a vacuole, and the cysts. After several days these tended to be found towards the periphery of the plates as amoebae migrate from the site of inoculation.

Sensitivity testing was initially carried out using a tube method as previously described.[1] Further testing was performed by adapting the tube method for microtitre plates. The acanthamoebae were washed off a non-nutrient agar plate with PBS and mixed with a turbid suspension of live *E. coli*. The mixture was spread over each of four 9 cm diameter non-nutrient agar plates and incubated at 37°C (or 25°C) for three days. Acanthamoeba isolates were washed off these plates with PBS, rubbing the surface with a loop, and counted in a

Neubauer chamber. the concentration was adjusted to 5×10^4 trophozoites and cysts (mixed) per ml PBS. Drugs were prepared by dilution from an ampoule, or by weight from powder, at twice the required initial concentration and titrated in 'double-dilutions' in 100 microlitre amounts in a microtitre plate. 100 microlitres of an amoeba/*E. coli* mixture were added to each well as was a control containing 100 microlitres of amoebae suspended in PBS. Plates were incubated at 37°C (or 25°C) for three days and examined with an inverted microscope. Numbers of amoebae were compared with the control to estimate the minimum inhibitory concentration (MIC); wells in which amoebae were considered to be multiplying were subcultured to non-nutrient agar plates (seeded with *E. coli*) which were incubated for three days, to estimate the minimum amoebicidal concentration (MAC) for each drug. Drug sensitivities were established for three isolates obtained from this patient in January, February and March 1988 (Table I).

Results

The acanthamoeba isolate in January 1988 grew at 37°C. Following treatment with the topical arsenical compound R6/56, isolates in February and March 1988 failed to grow at 37°C but did so at 25°C.

Inhibitory and amoebicidal concentrations of the drugs tested are given in Table I. The February and March isolates developed resistance to Brolene at R6/56 but otherwise had similar MICs to the January isolate.

Discussion

Medical cure of corneal infection due to *Acanthamoeba sp.* can be achieved with combined topical Brolene and neomycin therapy,[1] but requires early diagnosis and commencement of therapy. It may also depend on low minimum inhibitory concentrations; for diamidines<5 μg/ml and for neomycin <20 μg/ml. Various other types of drugs (antiprotozoal, antitrypanosomal and antifungal) have been tested for activity against acanthamoebae and a range of MICs have been demonstrated.[1] This range may explain the empirical requirement for combined therapy. Development of new drugs is hampered by lack of detailed knowledge of metabolic pathways in acanthamoebae, but progress has recently been reported in identifying key enzymes (S-adenosylmethionine decarboxylase) in polyamine synthesis. This is inhibited by dibromopropamidine, resulting in encystment. *In vitro* tests show that this inhibition can be reversed by putrescine or spermidine, hence maintaining the amoebae in trophozoite form which is more susceptible to drug therapy.[2]

Neomycin was witheld after one month in our patient due to epithelial toxicity. Brolene and dexamethasone were continued, but recurrence supervened and acanthamoeba were isolated at 37°C. A trial of topical R6/56 (an arsenical compound) was started. This is typanocidal and filaricidal; its melaminyl radical was intended to reduce the hazard of optic nerve atrophy which was a problem with the

Table I *Drug sensitivities of Acanthamoeba isolates*

Drug type	*Chemical group*	*Name*	*MIC μg/ml: trophozoites () = MAC: cysts*		
			Jan	*Feb**	*Mar**
Antibacterial and Antiprotozoal	Diamidines	Dibromopropamidine	<1	>50	>50
	Diamidines	Pentamidine isethionate	—	—	22
Antitrypanosomal	Phenanthridinium	R11/29	37 (37)	4.7 (9.4)	4.7 (3.8)
Antibiotic	Aminoglycoside	Paromomycin	9.4 (38)	9.4 (38)	9.4 (38)
Antibiotic	Polypeptide	Polymyxin B	—	—	150
Antimicrobial	Arsenical	R6/56	2.5 (10)	>150	>150
Antifungal	Imidazole	Clotrimazole	7 (7.5)	9.4 (19)	19 (75)

Results are given for three isolates obtained in January, February and March 1988
*Temperature-sensitive isolates

early arsenical drugs Atoxyl and Tryparsemide.[3] The initial concentration was 100 μg/ml (0.1%), later increased to 500 μg/ml to exceed an MIC of 2-5 μg/ml (for trophozoites) and an amoebicidal concentration of 10 μg/ml (for cysts). After 4 weeks' therapy, acanthamoebae were still isolated from the cornea but now failed to grow at 37°C. They did grow at 25°C and were found to be resistant to both R6/56 and Brolene. The question arises whether this temperature-sensitive mutation arose as a result of Brolene therapy alone, or due to R6/56 or both? The mutant continued to be isolated from corneal cultures for six months.

Clinical resolution of Acanthamoeba keratitis may take some weeks during which time an epithelial defect may persist and because the eye may be vulnerable to secondary infection, recurrent infiltrates should be investigated by corneal scraping for culture. In this case, Acanthamoebae were grown at 37°C at the time of the initial recurrence. Isolates subsequently failed to grow at 37°C as they became temperature-sensitive. 'Negative' cultures of corneal scrapes may occur in Acanthamoeba keratitis when the amoebae have migrated from the superficial stroma and have invaded the deeper stroma.[4] They may also indicate other, secondary pathogens or mutant amoebae. Hence corneal biopsy should be considered in indolent keratitis to obtain specimens from the deep stroma and isolation should be attempted at 25°C if amoebae fail to grow at 37°C. Molecular biology is likely to provide rapid identification of strains in the future, possibly distinguishing resistant mutants.

Therapy with imidazoles and aminoglycosides failed in this case, although sensitivity tests revealed MICs in the range 7–9μg/ml. Paromomycin in combination with benzethonium chloride did appear effective in controlling recurrence in the second PK in the left eye. Benzethonium chloride combined with paromomycin is effective treatment for cutaneous Leishmaniasis.[5] The right eye required additional R11/29[3] (a phenanthridinium compound active against pentamidine-resistant protozoa) and clotrimazole to which the mutant remained moderately sensitive. Combination therapy appears important for drugs with moderate to high MICs.

Surgery appears to have made a therapeutic contribution in the left eye. Fresh corneal tissue may attract residual amoebae from peripheral host cornea. It has been noted that amoebae are associated with keratocyte depletion[6] and new donor corneal tissue presents a new source of keratocytes for amoebic ingestion. Relocation of amoebae within the first PK may be considered to have facilitated removal of the amoebae since the graft was replaced after three months. Surgery can only be considered adjunctive treatment. It may promote transformation to trophozoite by presenting fresh keratocytes, thereby increasing susceptibility to topical therapy.

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