Activities of Therapeutic Agents and Myristamidopropyl Dimethylamine against *Acanthamoeba* Isolates

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The activities of therapeutic agents and myristamidopropyl dimethylamine (MAPD) against *Acanthamoeba* strains recalcitrant to medical therapy were studied. MAPD minimum cysticidal concentrations were 6.25 to 25 µg/ml; 10 to 30 µg/ml gave at least a 3-log cyst kill after 6 h, and 50 and 100 µg/ml gave at least a 3-log cyst kill within 2 and 1 h, respectively.

Keratitis caused by the free-living amoeba *Acanthamoeba* is one of the most difficult ocular infections to manage successfully due to the resistance of the organism's cyst stage to most antimicrobial agents at concentrations tolerated by the cornea (10, 11). Successful medical therapy has been reported using topical application of the diamidine compound propamidine isethionate alone or in combination with the aminoglycoside neomycin (16) and with antifungal imidazole derivatives such as miconazole and itraconazole (9). However, the rationale for the use of many of these agents is not supported by *Acanthamoeba* sensitivity testing, which has shown that while aminoglycoside and imidazole agents are active against the trophozoites, the cysts are markedly resistant (11, 12, 16).

The introduction of the polymeric biguanide polyhexamethylene biguanide (PHMB) as a 0.02% topical solution, either alone or in combination therapy, has improved the prognosis for acanthamoeba keratitis and studies have confirmed its in vitro and in vivo superiority over other treatments (4, 6, 11, 12). Similar success has also been recorded with topical chlorhexidine digluconate (0.02%), which has also been shown to have good cysticidal activity (15). The propamidine homologue hexamidine di-isethionate has been shown to have greater cysticidal activity than propamidine and has been used alone and in combination therapy with PHMB or chlorhexidine to treat acanthamoeba keratitis (2, 14).

Despite these advances in the treatment of acanthamoeba keratitis, failures still occur, necessitating prolonged and intensive medical therapy, surgical intervention, and the possible permanent loss of visual acuity or enucleation of the eye (1, 6, 11, 13). A new antimicrobial agent (myristamidopropyl dimethylamine [MAPD]) present in Opti-Free Express Multi-Purpose Disinfecting solution for contact lenses has been shown to exhibit anti-acanthamoeba activity (3). To address the need for improved therapeutic agents, we have studied the cysticidal activity of MAPD against acanthamoeba keratitis strains.

Nine clinical isolates of *Acanthamoeba*, eight of which showed a poor response to conventional medical therapy, were

studied. For the minimum cysticidal concentration (MCC) studies, cysts were obtained from prolonged incubation of the trophozoites on nonnutrient agar-*Escherichia coli* in air at 32°C for 7 days (10). For the time-kill studies with MAPD, cysts were obtained by growing trophozoites in the semidefined axenic medium supplemented after autoclaving with filter-sterilized 50 mM MgCl₂ (#6-Mg medium). Trophozoites were inoculated at 10⁵/ml in 100 ml of medium in an upright 175-cm² disposable tissue culture flask for incubation at 32°C in air with shaking at 100 rpm for 7 days. The cysts were recovered from the flask, washed three times with 1/4 strength Ringer's solution by centrifugation at 1,000 × g for 10 min, and stored at 4°C for testing within 14 days.

The following therapeutic and experimental agents were studied: 0.1% propamidine isethionate (Brolene Rhone-Poulenc, Rorer, England), 0.1% hexamidine di-isethionate (Desomedine, Chauvin Laboratory, Montpellier, France), 0.02% PHMB (Moorfields Eye Hospital Pharmacy, London, England), 0.02% chlorhexidine digluconate (Moorfields Eye Hospital Pharmacy), and 0.01% MAPD (Alcon Laboratories Inc., Fort Worth, Tex.).

The MCC assays were performed as described previously (7, 8). The MCC was defined as the lowest concentration of test compound that resulted in no excystment and trophozoite replication after 24 h of exposure. The kinetics of MAPD activity against *Acanthamoeba polyphaga* (Ac-Ros) #6-Mg cysts were determined as described previously (8). MAPD was dissolved and diluted in 2 mM Tris-HCl, pH 7.2, for testing at 5 to 100 µg/ml. Assays were performed in 20-ml glass universal bottles with 0.1% Tween 80 used as the neutralizer of the MAPD. The effect of test bottle material on MAPD activity against Ac-Ros was investigated in glass, polypropylene, and polystyrene. The activity of MAPD in glass at 50 µg/ml was then studied against the cysts of all *Acanthamoeba* strains.

The number of surviving cysts in the time-kill studies was calculated as described previously for *Acanthamoeba* (8). The reduction in organism viability was plotted as the change in log viability for each time point compared to zero time viability. Statistical analysis was performed using one-way analysis of variance or a paired *t* test.

The MCCs for the therapeutic agents and MAPD against the nine strains are shown in Table 1. The difference in activity

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TABLE 1. *Acanthamoeba* MCCs and kill rate by MAPD

Strain	MCC ($\mu\text{g/ml}$)					MAPD (50 $\mu\text{g/ml}$) log cyst kill ^a (mean $\pm$ SEM) by:	
	Propamidine isethionate	Hexamidine di-isethionate	PHMB	Chlorhexidine digluconate	MAPD	1 h	2 h
Ac-1	>500	500	1.60	31.25	12.50	3.28 $\pm$ 0.16	3.28
Ac-2	500	250	1.60	15.625	12.50	3.38 $\pm$ 0.08	3.38
Ac-3	>500	500	3.10	25	25	2.90 $\pm$ 0.42	2.90
Ac-4	500	250	3.10	31.25	6.25	3.36 $\pm$ 0.31	3.36
Ac-5	500	500	3.10	31.25	25	1.49 $\pm$ 0.29	4
Ac-Ros	500	250	3.10	25	25	2.52 $\pm$ 0.35	4
Ac-6	>500	250	3.10	25	12.50	2.30 $\pm$ 0.30	4
Ac-7	>500	250	6.75	25	12.50	2 $\pm$ 0.83	3.70
Ac-8	500	500	3.10	31.25	12.50	1.70 $\pm$ 0.23	3.70
Total (mean $\pm$ SEM)	≥ 500	361 $\pm$ 44	3.2 $\pm$ 0.5	26.7 $\pm$ 17.4	16.0 $\pm$ 2.4	2.55 $\pm$ 0.2	3.6 $\pm$ 0.1

^a Total kill of the cyst challenge inocula was obtained by 2 h for all strains.

between propamidine and hexamidine against the strains was statistically significant ($P < 0.001$) but that between PHMB, chlorhexidine, and MAPD was not ($P > 0.05$). The kinetics of MAPD killing of Ac-Ros cysts with 5 to 100 $\mu\text{g/ml}$ are shown in Fig. 1. Maximum log kill with 5 $\mu\text{g/ml}$ was 1.44 ± 0.17 log (mean $\pm$ standard error of the mean) at 8 h; with 10 $\mu\text{g/ml}$ was 4.35 ± 0.09 log at 8 h; with 20 $\mu\text{g/ml}$ was 3.38 ± 0.08 log at 6 h; with 30 $\mu\text{g/ml}$ was 4.11 ± 0.11 log at 6 h or 3.62 ± 0.56 log at 4 h; with 50 $\mu\text{g/ml}$ was 4.08 ± 0.34 log at 2 h; and with 100 $\mu\text{g/ml}$ was 4.23 ± 0.11 log at 1 h. MAPD at 50 $\mu\text{g/ml}$ gave total kill of the cyst challenge (3 to 4 log) within 1 h for four of the strains and by 2 h for all nine strains (Table 1).

Loss of MAPD activity was observed when assays were performed in polypropylene and polystyrene tubes. In glass, 10 $\mu\text{g/ml}$ gave 3.44 ± 0.48 log kill of Ac-Ros at 6 h compared to 1.57 ± 0.37 log in polypropylene and 1.96 ± 0.14 log in polystyrene. The decreased activity in polypropylene and polystyrene compared to that in glass is significant at this time point and also at 8 h for polypropylene but not for polystyrene ($P < 0.05$).

For this study, in vitro drug sensitivity testing showed the strains to be resistant to propamidine isethionate and, to a

lesser extent, hexamidine di-isethionate. The greater cysticidal activity of hexamidine di-isethionate than of propamidine isethionate has been noted previously, and the agent has been used in the successful treatment of the infection (2, 14). However, all strains were sensitive to PHMB, chlorhexidine digluconate, and MAPD. Accordingly, there is a poor correlation between in vitro MCC findings and patient response to these therapeutic agents, as has been observed previously (7).

Although the introduction of PHMB and chlorhexidine has dramatically improved the treatment of *acanthamoeba* keratitis, relapse with continued culture-positive isolation of *Acanthamoeba* occurs in up to 10% of patients (6, 7, 13). MAPD is a cationic amidoamine also known as stearamidopropyl dimethylamine, *N*-[3-(dimethylamino) propyl] octadecanamide, or *N,N*-dimethyl-*N'*-tetradecanoyl-1,3-propylenediamine. Here, MAPD in 2 mM Tris-HCl, pH 7.2, showed a low MCC for all strains (6.25 to 25 $\mu\text{g/ml}$) and at 50 $\mu\text{g/ml}$ gave total cyst kill within 1 to 2 h following a challenge inoculum of 3 to 4 log organisms in the time-kill studies.

The mode of action of MAPD and therapeutic agents against *Acanthamoeba* is not known. In bacteria, PHMB and chlorhexidine cause cytoplasmic membrane damage, resulting in an irreversible loss of essential cellular components following binding to the cell wall (5). The alternative chemical structure and low molecular weight of MAPD (molecular weight, 300) compared to those of PHMB (average molecular weight, 2,340) and chlorhexidine digluconate (molecular weight, 898) might permit better penetration into the cornea to achieve therapeutic levels. The findings of this study indicate that MAPD is an effective *Acanthamoeba* cysticidal compound and may represent an improved agent in the treatment of *acanthamoeba* keratitis and, possibly, other forms of microbial keratitis.

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FIG. 1. Time-kill studies with 5 to 100 μg of MAPD per ml against Ac-Ros cysts. ●, 5 $\mu\text{g/ml}$; ■, 10 $\mu\text{g/ml}$; ▲, 20 $\mu\text{g/ml}$; ○, 30 $\mu\text{g/ml}$; □, 50 $\mu\text{g/ml}$; △, 100 $\mu\text{g/ml}$.

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