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Effects of Magainins on Ameba and Cyst Stages of *Acanthamoeba polyphaga*

F. L. SCHUSTER[1]* AND L. S. JACOB[2]

Biology Department, Brooklyn College, Brooklyn, New York 11210,[1] and Magainin Pharmaceuticals, Inc., Plymouth Meeting, Pennsylvania 19462[2]

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Amebic keratitis produced by *Acanthamoeba* spp. is an increasingly important ocular infection in extended-use contact lens wearers. Problems associated with the infection are compounded by the lack of effective and well-tolerated chemotherapeutic agents. The magainins, a group of naturally occurring and synthetic membrane-active peptide compounds, have been shown to be active in vitro against a clinical isolate of *Acanthamoeba polyphaga*. Two magainins tested extensively had minimal inhibitory and minimal amebicidal values of 20 and 25 μg/ml for magainin MSI-103 and 25 and 40 μg/ml for magainin MSI-94, respectively. Both amebastatic and amebicidal activities are enhanced by combining the magainins with silver nitrate (200 μg/ml) and/or other marginally effective antimicrobial agents. These combinations have activity against both trophic and cystic stages in the *Acanthamoeba* life cycle and have promise as antimicrobial agents in the treatment of amebic keratitis.

Acanthamoeba keratitis, a potentially sight-threatening disease, has been seen in recent years with increasing frequency (23). Initially recognized as a consequence of corneal trauma (12), recent infections have been increasingly associated with daily-wear soft contact lenses, hard lenses, gas-permeable hard lenses, and combined hard-soft lenses. Any departure from recommended contact lens care which exposes the lens or the lens case to a nonsterile environment contaminated with *Acanthamoeba* spp. can lead to clinical infection (14, 21). Pain and recurrent epithelial breakdown often accompany the course of the disease. *Acanthamoeba* keratitis is frequently misdiagnosed as a herpes simplex virus or bacterial keratitis, thus delaying the onset of appropriate therapy (4, 11, 20, 27).

The life cycle of *Acanthamoeba* spp. consists of the trophic ameba alternating with a dormant, thick-walled cyst. During infection, both cysts and trophozoites are found within the cornea (12, 15). Unfavorable conditions can induce the encystment of trophozoites, rendering the infection more difficult to eradicate because of the resistance of the cyst to many of the antimicrobial agents effective against the ameboid stage (19, 24).

Corneal infections caused by *Acanthamoeba* spp. have been difficult to heal (4). Medical treatment often fails, and recurrent infections following therapeutic corneal transplantation have been common. There is need for an effective antimicrobial agent, one that is able to kill both trophic and cystic stages of the ameba and that is well tolerated in the eye without causing undue discomfort or toxicity. Magainin host defense peptides, originally isolated from the African clawed frog (32), possess a unique membrane-targeted mechanism of action which likely includes altering ion conductances across membrane barriers (1). They are active against a broad range of gram-positive and gram-negative bacteria, including *Staphylococcus aureus* and *Pseudomonas aeruginosa* (17), and possess significant antifungal, antiviral, and antiparasitic activity (32, 33). We now demonstrate that magainins alone and/or in combination with other antimicrobial agents produce amebastatic and amebicidal effects on trophic populations of *Acanthamoeba polyphaga*, an important etiologic agent of amebic keratitis (15, 27, 30). Additionally, they either delayed cyst germination or were cysticidal, suggesting that magainins may have clinical utility in the treatment of *Acanthamoeba* keratitis.

(Parts of this study were presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy held in Atlanta, Ga. in October, 1990 [25].)

MATERIALS AND METHODS

Organisms and growth. The organism used in this study was *A. polyphaga* (ATCC 30461), isolated from a case of amebic keratitis (12). The amebas were grown axenically at 30°C in *ppyg* medium, which consists of Oxoid Proteose Peptone (2%, wt/vol)–yeast extract (0.5%, wt/vol)–glucose (0.5%, wt/vol) (pH 7.2). Corning tissue culture flasks (25 cm^2) were used for growth of cells for stock cultures and experiments. Logarithmic-phase cultures of trophic amebas (generation time of ca. 11 h) were used for drug testing. Replicate cell counts were carried out with a Coulter Counter (model Z_F). Data included on growth and antimicrobial effects are based on at least two or more experiments.

Magainin antimicrobial agents. The two magainins that were tested most extensively, MSI-103 and MSI-94 (obtained from Magainin Pharmaceuticals, Plymouth Meeting, Pa.), are basic linear peptides composed of 21 and 22 amino acids, respectively. The peptides were prepared by solid-phase peptide synthesis, purified by reverse-phase and ion exchange high-performance liquid chromatography, and converted to acetate salt. MSI-103 is an analog of a peptide extracted from the skin of *Xenopus laevis*; MSI-94 is an analog of magainin II (32). Both peptides are able to form an alpha-helical structure in the presence of biological membranes and can form ion channels in some membranes (18).

Drug treatment. Working stock solutions (2 mg/ml) of the magainins were prepared from powdered drug by using

* Corresponding author.

distilled water as the solvent. Stocks were filter sterilized with Gelman 0.22-μm-pore-size filters and stored at refrigerator temperatures. For experimental drug testing, amebas were added to *ppyg* culture medium to give an initial cell concentration of ca. 10,000 amebas per ml. Appropriate aliquots of the magainin concentrate were added to the growth flasks, and the flasks were incubated at 30°C. Samples (0.5 ml) were removed from the growth flasks for counting at 24-h intervals, usually over a period of 5 days. In those experiments in which the combined action of drugs was being tested, the second antimicrobial agent was added to the growth flask at the time of inoculation with the ameba suspension. Compounds tested for antiamebic activity as well as for synergy with the magainins included silver nitrate (Fisher), bacitracin (Sigma), Brolene eye drops (containing 0.1% [wt/vol] propamidine isethionate and 0.01% [vol/vol] benzalkonium chloride [May & Baker]), erythromycin (Sigma), ciprofloxacin (Miles Pharmaceuticals), gramicidin S (Sigma), ketoconazole (Janssen Research Foundation), neomycin sulfate (Sigma), pentamidine isethionate (May & Baker), and polymyxin B (Sigma). In order to determine whether a particular magainin concentration was amebastatic or amebicidal, cells were washed free of medium containing antimicrobial agents and cultured in fresh *ppyg* medium for up to 21 days. The lowest concentration inhibitory to growth was noted as the MIC, while the lowest concentration that killed cells was noted as the minimal amebicidal concentration (MAC). Appropriate concentrations of potential synergists for testing in conjunction with magainins were determined by preparing dilution series of the antimicrobial agent at halving concentrations starting at 100 μg/ml. Amebicidal or amebastatic effects of these drugs on trophic amebas were noted.

***Acanthamoeba* cysts.** The magainins were also evaluated for their abilities to inhibit germination or kill cysts of *A. polyphaga*. Cysticidal properties were assayed initially by exposing washed cysts produced in bacterized cultures (using *Klebsiella pneumoniae* as the bacterial food source) to drugs for time periods of up to 48 h and then plating the cysts on agar in the presence of *K. pneumoniae* to look for cyst germination and growth of excysted amebas. In order to minimize the effect of bacteria, subsequent studies made use of cysts induced to form from axenic ameba populations by washing and suspending trophozoites in 50 mM magnesium chloride (9), which produced better than 90% encystation over a 5-day period. These cysts were tested with magainins and other compounds in *ppyg* medium, in which both germination of cysts and growth of amebas were assayed.

RESULTS

Effect of magainins on growth. Tests of MSI-94 and MSI-103 were carried out at a range of 1 to 50 μg/ml. The effects on growth of these two compounds are seen in Fig. 1a and b. Low concentrations (1 to 10 μg/ml) of both magainins were initially inhibitory to the extent of ca. 99% for a 2- to 3-day period compared with the drug-free control, but growth of amebas resumed with cells appearing morphologically normal. Mid-range concentrations (15 to 30 μg/ml) were not only inhibitory but appeared to cause cellular lysis, as indicated by a decrease in numbers of trophic amebas and the concommitant appearance of granular debris in the growth flasks. Concentrations at the high end of the range (30 to 50 μg/ml) caused extensive lysis, verified by visual inspection of the growth flasks with a tissue culture microscope. At both moderate and high concentrations, the amebas appear shrunken and irregular in shape. By visual inspection alone, it is not possible to determine whether the amebas are dead or alive, since no pseudopodial or vacuolar activity is evident. Upon replacement of drug-containing medium with fresh medium, several days elapse before cells assume a more normal appearance and start proliferating.

MICs and MACs were in the mid-range concentrations for both magainins. For MSI-94, the MIC and MAC were 25 and 40 μg/ml, respectively. For MSI-103, the MIC and MAC were closer, at 20 and 25 μg/ml, respectively. In either case, it was not possible to determine the MIC and MAC until the cells were washed free of magainin-containing medium and cultured in fresh *ppyg* growth medium. Recovery of amebas took 7 to 10 days; when MICs were being determined, it took as long as 21 days in some instances.

Drug combination experiments. A variety of other antimicrobial and chemical agents were tested as potential synergists for use with magainins. These included the compounds listed in Table 1, where both MICs and MACs for each compound are indicated. These agents were tested with the two magainins. Most promising of these compounds were silver nitrate, ketoconazole, propamidine isethionate (as the commercial preparation Brolene), gramicidin S, and neomycin sulfate.

Because of its efficacy in the treatment of eye infections (8), silver nitrate was extensively tested with the two magainins. The results of some of these tests are illustrated in Fig. 2a and b. Silver nitrate at 200 μg/ml was only slightly inhibitory, but in combination with the magainins (used at concentrations of 5 to 20 μg/ml) it was either amebastatic or amebicidal. In these experiments, magainin concentrations were kept low in order to better demonstrate the effects of the drug combinations. The degree of inhibition observed was proportional to the silver nitrate concentration in the medium. Silver nitrate at 50 μg/ml gave ca. 5% inhibition; inhibition increased to 28 and 99% with silver nitrate concentrations of 100 and 200 μg/ml, respectively (Fig. 3). The effect produced upon the trophozoites was either amebastatic or amebicidal, probably reflecting the influx of silver nitrate.

The effects of combinations of magainins and other antimicrobial agents on the growth of trophic amebas are shown in Fig. 4a and b. Ketoconazole at a sub-MIC level of 25 μg/ml combined with magainins at 20 μg/ml was amebicidal (Fig. 4a). Other compounds tested that showed promise when used in combination include gramicidin S, neomycin, pentamidine isethionate, and polymyxin B (Fig. 4b). Brolene, an over-the-counter eye drop preparation (available in the United Kingdom) containing propamidine isethionate, was amebicidal at 1% (vol/vol) in combination with the magainins. The preservative benzalkonium chloride in Brolene had a slight amebastatic effect over a period of 2 days, which perhaps enhanced the killing activity of the preparation. Little or no additive effect against *A. polyphaga* was seen with bacitracin, ciprofloxacin, and erythromycin.

Cysts. The two magainins were tested for their abilities to kill the encysted stage of *A. polyphaga*. Initially, testing was performed by obtaining cysts from cultures raised on the bacterium *K. pneumoniae* (see Discussion for rationale). These are referred to as bacterized cysts. Subsequent efforts utilized cysts induced to form from axenic amebas by washing and suspension in 50 mM $MgCl_2$ (referred to as axenic cysts). Cysts produced in bacterized cultures exhib-

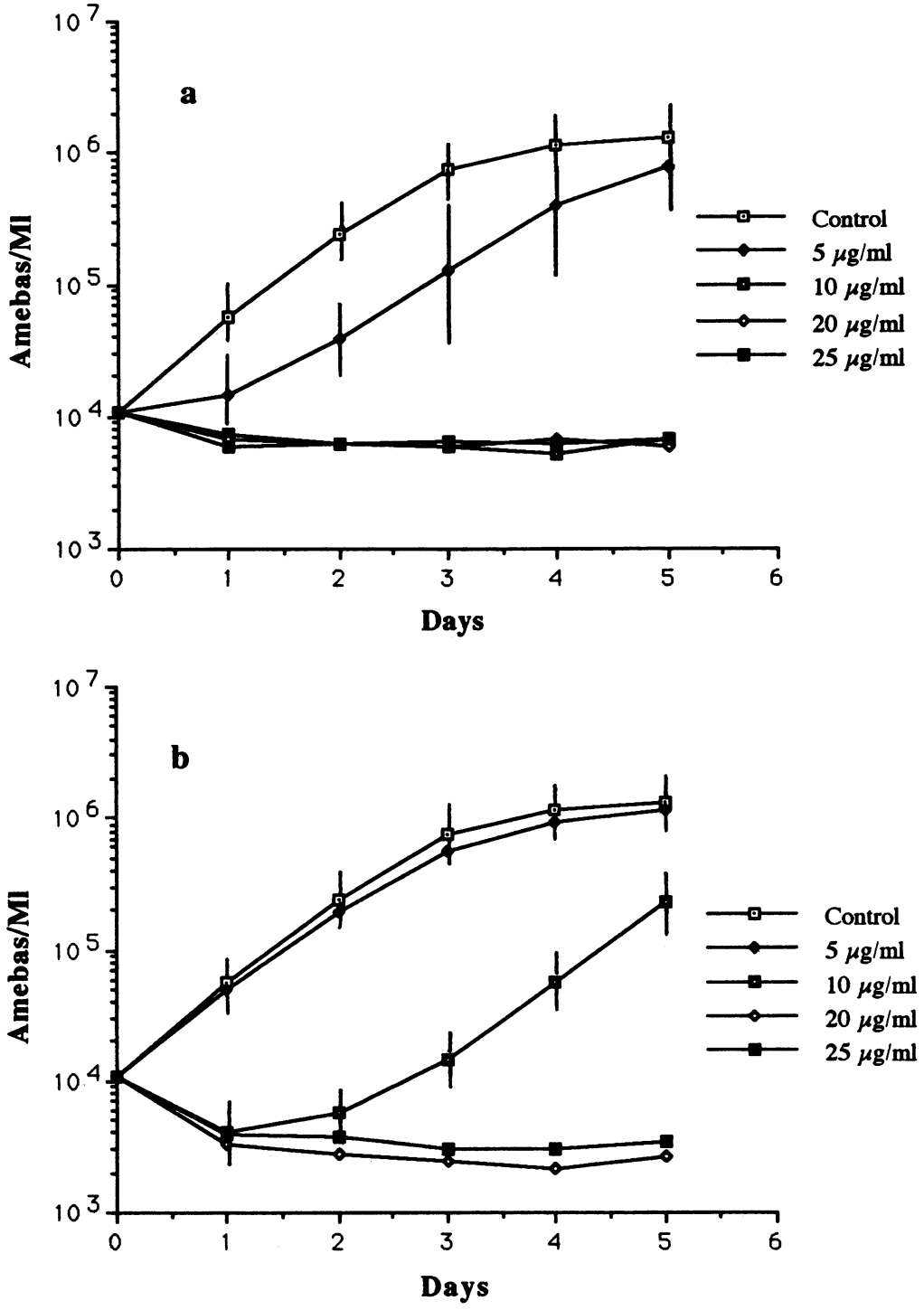

FIG. 1. Growth responses of *A. polyphaga* trophozoites to various concentrations of the magainins MSI-94 (a) and MSI-103 (b). For MSI-94, the MIC and MAC were 25 and 40 μg/ml, respectively; comparable values for MSI-103 were 20 and 25 μg/ml. Effects of higher concentrations are not included in the graphs. Growth of the control *Acanthamoeba* population represents the average for normal growth in 10 different experiments and is used as the control in all the graphs for trophic growth. Ameba growth is represented as amebas per milliliter. Error bars represent ± 1 standard deviation.

ited more variability in response to antimicrobial treatments. In general, however, exposure of bacterized cysts to magainin plus silver nitrate for 24 to 48 h was cysticidal. Cysts were more resistant than trophozoites to equivalent magainin concentrations.

Two factors were studied in these experiments: (i) cyst germination in the presence of magainin, and (ii) proliferation of the excysted amebas. The results of the use of MSI-103 are shown in Fig. 5. MSI-103 by itself inhibited the germination and growth of amebas. The addition of silver

TABLE 1. In vitro drug sensitivity of trophozoites of an *A. polyphaga* isolate

Antimicrobial agent	MIC (μg/ml)	MAC (μg/ml)
Bacitracin	25	40
Erythromycin	25	50
Ciprofloxacin		>100
Gramicidin S	3	6
Ketoconazole	50	100
Neomycin sulfate	25	50
Pentamidine isethionate	12.5	25
Polymyxin B	25	50
Silver nitrate		>100

nitrate at 200 μg/ml, which by itself had a slight inhibitory effect on cysts, was cysticidal in combination with MSI-103. The cysticidal effect was demonstrated by harvesting and washing cysts (at day 7) free of the magainin-containing medium and suspending them in fresh growth medium.

DISCUSSION

Acanthamoeba infections are insidious and generally refractory to antimicrobial treatment. Those of the central nervous system (granulomatous amebic encephalitis) have been recognized in compromised hosts, most recently in AIDS patients (15, 19). Those of the cornea are mostly the result of lapses in lens care hygiene or the use of contaminated water in the preparation of lens cleaning solutions (13, 28). Although more readily diagnosed than granulomatous amebic encephalitis, amebic infections of the cornea have caused concern, especially because of the increasing popularity of extended-use contact lenses.

Reports in the literature have supported the in vitro or in vivo efficacy of a variety of antimicrobial agents in the treatment of *Acanthamoeba* keratitis. In the case of in vitro testing, these agents have included clotrimazole (29), 5-fluorocytosine (2), hydroxystilbamidine isethionate (2, 22), magainins I and II (7), pimaricin (16), trifluoperazine (26), and pentamidine and propamidine isethionate (13). Antimicrobial agents found to have efficacy in clinical applications have included oral itraconazole along with topical miconazole (11), topical clotrimazole (6), dibromopropamidine ointment along with propamidine and neomycin drops (30), propamidine isethionate as Brolene either alone (31) or in combination with topical neomycin-polymyxin B-gramicidin as Neosporin (20), and Neosporin with or without miconazole or ketoconazole (27). Often an antimicrobial agent effective in vitro is not well tolerated in the eye (30). Brolene, for example, has been reported to cause an adverse response in the eye and drug toxicity (20, 31). Another aspect of drug response to consider is the differential sensitivity of trophic amebas and cysts, the former being more sensitive than the latter (13, 19, 24). Cysticidal concentrations for various antimicrobial agents range from 10 to 1,000 times the dose effective against trophic amebas (22). Thus, the ideal agent for the treatment of *Acanthamoeba* keratitis should be well tolerated and have efficacy against cysts as well as trophozoites.

This report details effects of several magainins, a group of naturally occurring and synthetic membrane-active peptides, against ameboid and cyst stages of *A. polyphaga*. The magainin class of peptides is a family of antimicrobial compounds which form an amphipathic alpha-helix with hydrophobic residues on one side of the helix and hydrophilic residues on the other (10). Patch clamp analysis has demonstrated that magainin II, a peptide from frog skin, forms voltage-dependent ion channels in synthetic phospholipid bilayers (5). Unlike detergents, the magainin membrane activity appears reversible and selective (3). Synthesis of new derivatives via structure activity analysis has resulted in compounds that have enhanced anti-infective potency while not disrupting the membrane integrity of human cells. MSI-94 and MSI-103 are two such molecules. Structural modifications have resulted in a 10-fold increase in potency against gram-positive and gram-negative microbes (17), and similar enhanced activity was demonstrated in our experiments against *A. polyphaga*.

A particular advantage of the magainins is their presumed role in increasing cell membrane permeability (33). This has been exploited in the current study by combining magainins with several marginally effective antimicrobial agents—in particular, silver nitrate—to show increased activity in vitro against *A. polyphaga*. Silver nitrate has an established place in the pharmacopoeia as an effective and well-tolerated antimicrobial agent, used as a 1% solution in the treatment of neonatal ophthalmia (8). By combining silver nitrate with magainins, enhanced activity is seen in the responses against trophozoites and cysts. This may allow for use of both magainins and silver nitrate at concentrations lower than MACs, with retention of efficacy. The nature of the enhanced activity of silver when used in combination with magainins may relate to influx into the ameba cytoplasm as a result of increased membrane permeability. Other antimicrobial agents that show increased activity when used with magainins are the amidines (Brolene and pentamidine isethionate) and ketoconazole. Thus, the magainins are effective either alone or in various combinations with other antimicrobial agents.

At low concentrations, magainins inhibit growth to the extent of ca. 99% for a 2- to 3-day period, and then ameba growth resumes. It is assumed that resumption of growth follows the breakdown of the inhibitory peptide in the growth medium, perhaps enhanced by proteolytic enzymes elaborated by the amebas. Cellular disintegration is slight at moderate magainin concentrations (15 to 30 μg/ml) but is pronounced at higher concentrations (30 to 50 μg/ml), as indicated by particulate material in the growth flasks. In our attempts to resurrect magainin-treated cultures for MIC determinations, a week or more was required for recovery of trophozoites and, in some instances, as long as 3 weeks. These examples of recovery are significant in light of the difficulties encountered in the total elimination of infecting amebas by using antimicrobial agents alone and the need to resort to more aggressive treatment such as corneal transplant.

In this and other studies of drug effects on *Acanthamoeba* spp., a problem exists in distinguishing between live and dead cells following antimicrobial treatment. Except for the low drug concentrations in which the effects on cell morphology appear to be minimal, amebas tend to round up and cease moving. No vacuolar activity is seen in these cells, making it still more difficult to determine whether the cells are dead or alive. This is certainly a problem with the use of the Coulter Counter, which, of course, cannot distinguish dead from live cells, but it is also a problem with the use of a hemocytometer as a counting chamber. Thus, in this study, we have used cultivation of drug-treated cells in a drug-free medium as our criterion for deciding whether a given antimicrobial concentration or combination is amebicidal or

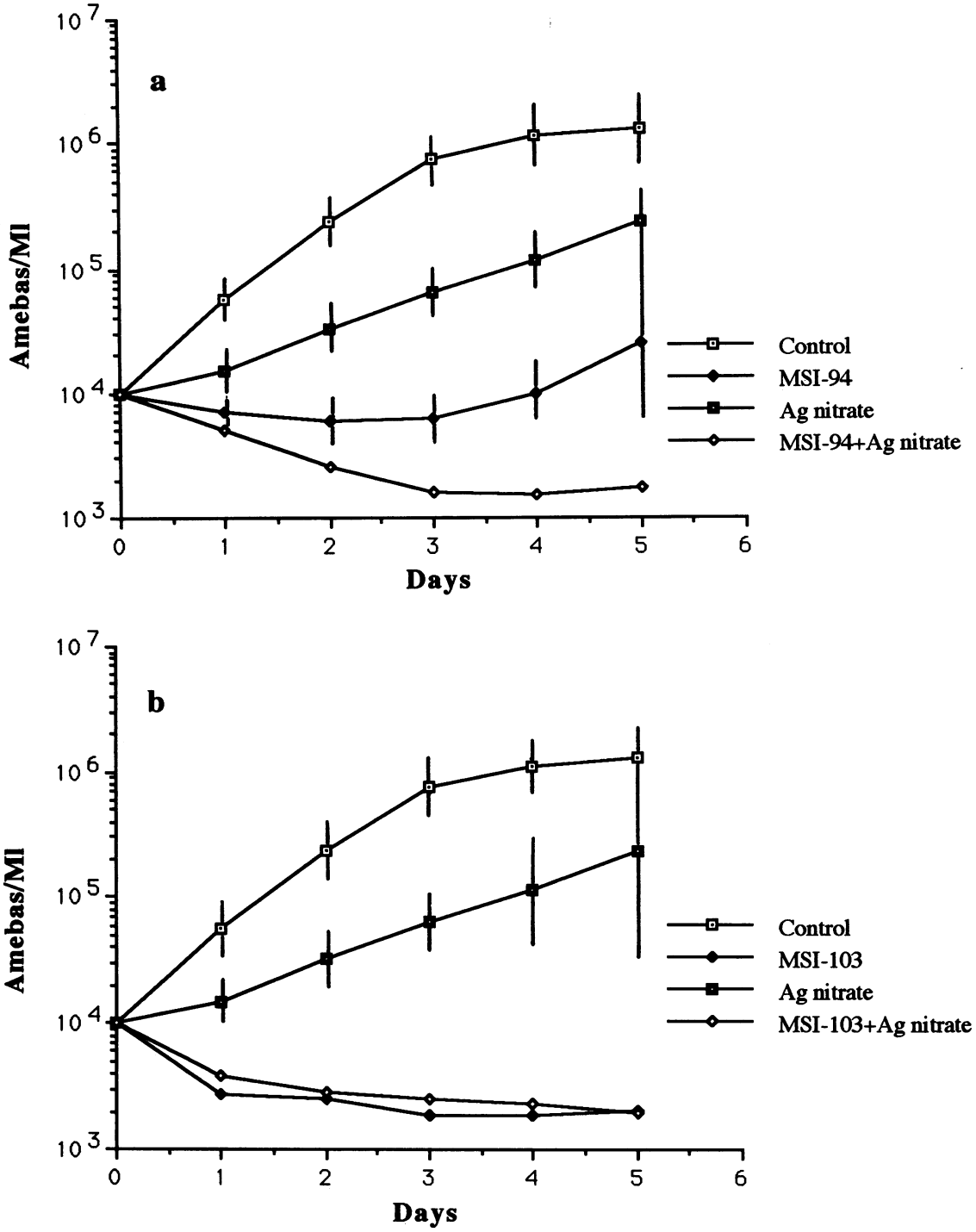

FIG. 2. Additive combinations of magainins MSI-94 and MSI-103 (20 μg/ml) and silver nitrate (200 μg/ml) tested against trophic amebas. The magainins were used at the MIC. Silver nitrate was used at a concentration that by itself was inhibitory but not amebicidal. The combinations, however, were amebicidal. Reducing the silver nitrate concentration to 50 or 100 μg/ml produced an amebastatic response in combination with these two magainin compounds. Error bars represent ± 1 standard deviation.

amebastatic. Absence of growth after 21 days is taken as an indication that a given concentration or combination of antimicrobial agents is amebicidal. This method of determining amebicidal versus amebastatic drug effects is not as precise as the definition used by the bacteriologists, according to which the MBC is that concentration that kills 99.9% of the bacterial population.

Amebic keratitis has been caused by several *Acanthamoeba* species, including *A. polyphaga*, *A. castellanii*, *A. hatchetti*, and *A. rhysodes* (15). This variety of etiologic

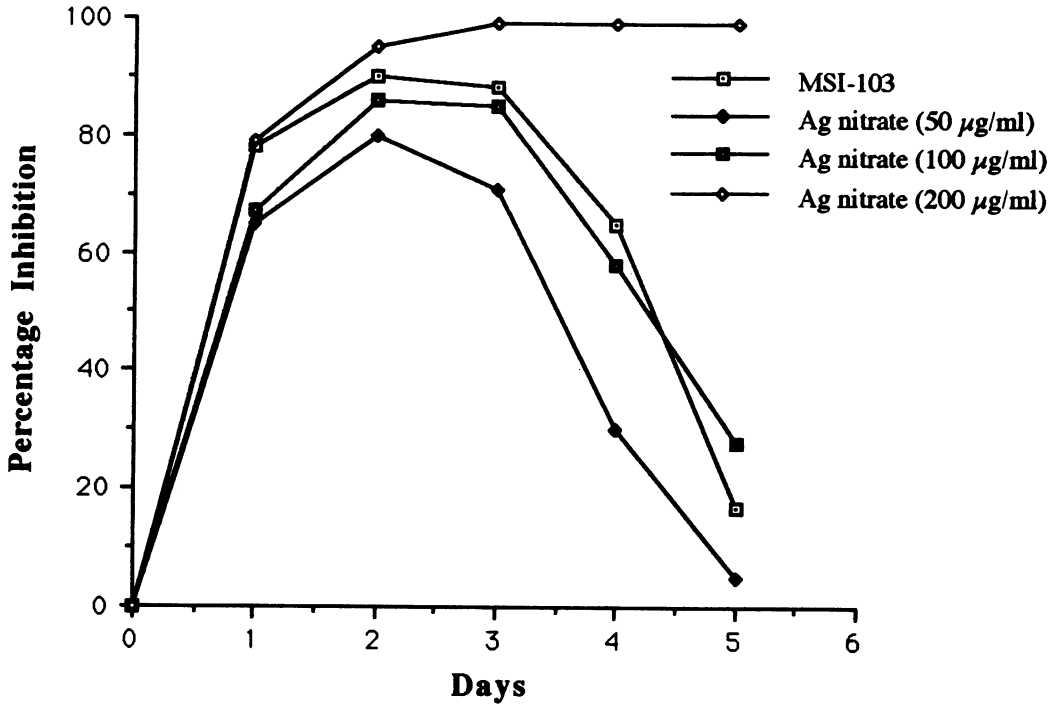

FIG. 3. Percent inhibition of growth of trophic amebas as a function of silver nitrate concentration. In this experiment, MSI-103 was used at 5 μg/ml, and the silver nitrate concentration varied (50, 100, or 200 μg/ml).

agents involved in keratitis is also the probable basis for differences and discrepancies encountered in antimicrobial efficacy. Considerable variations are noted in the tables of MICs and MACs for trophic amebas published in the literature. Much of this variation can be attributed to work done with different species of the genus *Acanthamoeba*, heterogeneity of strains of a single species, age of trophozoites or cysts being tested, and different methods used for cultivation and drug testing. In our study of the magainins as antiamebic agents, we have made use of viability as a criterion of effective amebicidal or cysticidal drug activity. This approach appears to give more meaningful results than microscopic inspection of drug-treated cultures. What appear to be nonviable amebas under the microscope can recover after several days or weeks. Feldman et al. (7), in testing magainins I and II against *A. castellanii*, a generally nonpathogenic species, found that considerably higher concentrations (>100 μg/ml) of magainins than those employed in the present study were required to inhibit or kill trophic amebas and that concentrations of >500 μg/ml were not cysticidal. As noted previously, magainin derivatives such as MSI-94 and MSI-103 are more potent than the magainin peptides originally isolated from frog skin.

A similar case can be made for testing antimicrobial agents against the cyst stage of *Acanthamoeba* spp. Antimicrobial agents effective against trophic amebas are not equally effective against cysts. Cysticidal concentrations of most drugs are considerably higher than amebicidal concentrations (13, 22, 24). Drug combinations are recommended as being more effective in destroying *Acanthamoeba* cysts than single drugs (24). Osato et al. (24) have found propamidine isethionate in combination with neomycin or paromomycin to show a marginal increase in efficacy. Others have found propamidine and paromomycin to be cysticidal at moderate concentrations (13, 22). In this study, a combination of magainin and silver nitrate appears to be as effective against cysts as against trophic amebas, and at similar concentration levels.

Encystment in axenic cultures of *Acanthamoeba* spp. is nominal, and those cysts that do form are often irregular in shape and less resistant to desiccation and prolonged storage. In cultures raised on bacteria (using a nonmucoid strain of *K. pneumoniae* as a suitable food organism) virtually all trophic amebas encyst, with the cysts having typical thick-walled morphology and being highly resistant to environmental stresses such as desiccation, elevated or refrigerator temperatures, storage, etc. In our studies, however, we have found that bacterized cysts gave erratic results in different experiments, with a particular drug combination being effective on one batch of cysts but not on the next. In an effort to circumvent this variability, cyst induction by use of magnesium chloride treatment of axenic cultures (9) was employed. Under these circumstances, better than 90% encystment occurred over 5 days. With storage of these cyst suspensions at 4°C, all amebas appeared to enter into the cyst state. Cysts prepared in this manner have remained viable after 1 year of storage. Thus, these induced cysts had several advantages over bacterized cysts: absence of bacteria as a variable, relative synchrony of cyst formation, and a standardized cyst population which can be tested over a period of time for comparative data. Cysts from these populations gave more reproducible results in drug-testing trials than did the bacterized cysts.

The results of this study encourage further testing of magainins and their derivatives for the ability to kill *Acanthamoeba* trophozoites and cysts in keratitis infections. While these results are preliminary, they suggest that magainins in combination with silver nitrate and/or other antimicrobial agents have in vitro activity against *Acanthamoeba*

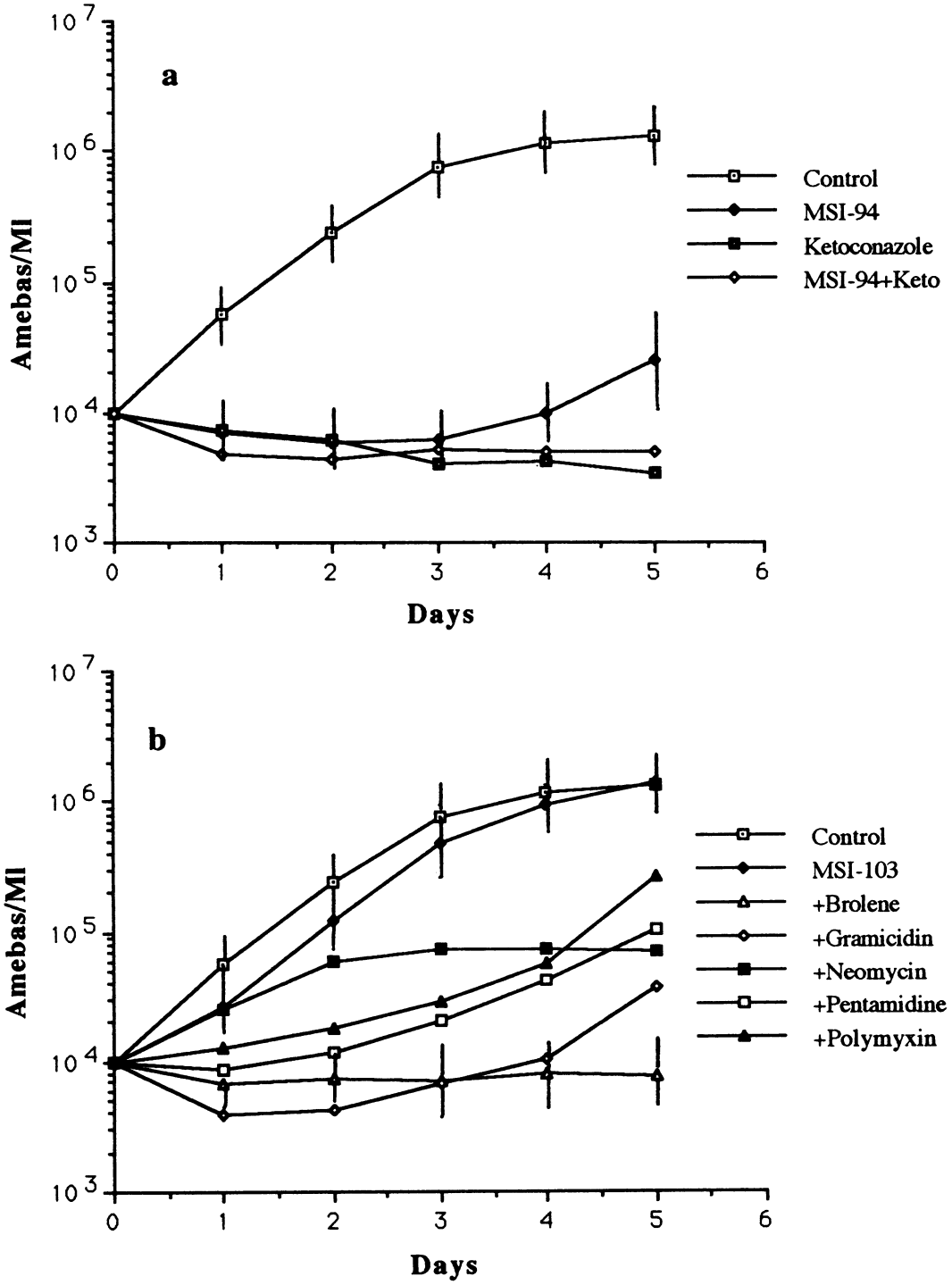

FIG. 4. Effect of magainins plus various other antimicrobial compounds tested against trophic amebas. (a) The combination of MSI-94 (20 μg/ml) and ketoconazole (25 μg/ml), which was amebicidal, is shown. Comparable results were obtained for the combination of MSI-103 and ketoconazole. (b) Combinations of MSI-103 (5 μg/ml) and other antimicrobial agents, including Brolene (1%, vol/vol), gramicidin S (3 μg/ml), neomycin (12.5 μg/ml), pentamidine isethionate (10 μg/ml), and polymyxin B (25 μg/ml). All of the combinations were amebastatic except for MSI-103 and Brolene, which was amebicidal. Error bars represent ± 1 standard deviation.

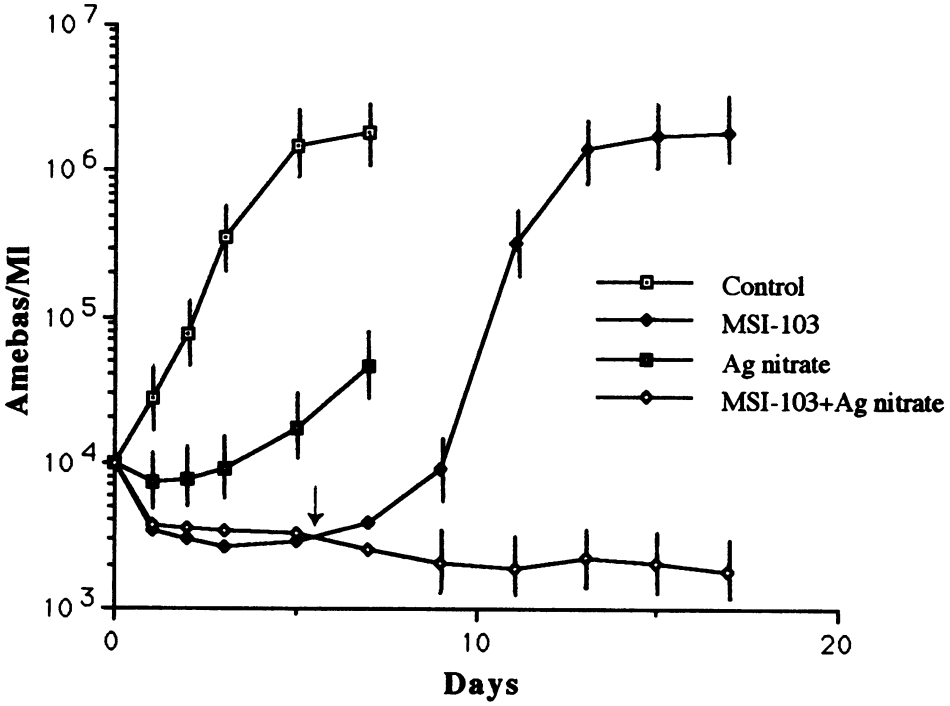

FIG. 5. Effect of MSI-103 (20 μg/ml) and silver nitrate (200 μg/ml) on excystation and growth of *A. polyphaga*. Cysts used were derived from axenic cultures by washing in 50 mM $MgCl_2$. The control curve represents normal cyst germination and growth of amebas in *ppyg* medium. Silver nitrate was inhibitory, as was MSI-103 by itself. At day 7 (arrow), the cysts were collected, washed free of inhibitor-containing medium, and suspended in fresh *ppyg* medium. This treatment activated cysts treated with MSI-103 alone, but it demonstrated that cysts exposed to magainin plus silver nitrate were no longer viable. Error bars represent ± 1 standard deviation.

spp. and that they may be useful in treating corneal infections caused by amebas.

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REFERENCES

1. **Berkowitz, B. A., C. L. Bevins, and M. A. Zasloff.** 1990. Magainins: a new family of membrane-active host defense peptides. Biochem. Pharmacol. **39**:625–629.
2. **Casemore, D. P.** 1970. Sensitivity of *Hartmannella* (*Acanthamoeba*) to 5-fluorocytosine, hydroxystilbamidine, and other substances. J. Clin. Pathol. **23**:649–652.
3. **Chen, H. C., J. H. Brown, J. L. Morell, and C. M. Huang.** 1988. Synthetic magainin analogues with improved antimicrobial activity. FEBS Lett. **236**:462–466.
4. **Cohen, E. J., C. J. Parlato, J. J. Arentsen, G. I. Genvert, R. C. Eagle, Jr., M. R. Wieland, and P. R. Laibson.** 1987. Medical and surgical treatment of *Acanthamoeba* keratitis. Am. J. Ophthalmol. **103**:615–625.
5. **Cruciani, R. A., E. F. Stanley, M. Zasloff, D. L. Levis, and J. L. Barker.** 1988. Magainin 2, a natural antibiotic from frog skin, forms anion-selective channels in lipid bilayer membranes. Biophys. J. **53**:9a.
6. **Driebe, W. T., G. A. Stern, R. J. Epstein, G. S. Visvesvara, M. Adi, and T. Komadina.** 1988. *Acanthamoeba* keratitis. Arch. Ophthalmol. **106**:1196–1201.
7. **Feldman, S. T., M. Speaker, and P. Cleveland.** 1991. Effect of magainins on *Acanthamoeba castellanii*. Rev. Infect. Dis. **13**(Suppl. 5):S439.
8. **Gilman, A. G., L. S. Goodman, T. W. Rall, and F. Murad (ed.).** 1985. Goodman and Gilman's the pharmacological basis of therapeutics, 7th ed. Macmillan Publishing Co., New York.
9. **Griffiths, A. J.** 1970. Encystment in amoebae. Adv. Microb. Physiol. **4**:105–129.
10. **Guy, H. R., and G. Raghanathan.** 1988. Structural models for membrane insertion and channel formation by antiparallel alpha-helical membrane peptides, p. 364–380. *In* A. Pullman, J. Jortner, and K. Pullman (ed.), Transport through membranes: carriers, channels, and pumps. Kluwer Academic Publishers, Boston.
11. **Ishibashi, Y., Y. Matsumoto, T. Kabata, R. Watanabe, S. Hommura, K. Yasuraoka, and K. Ishii.** 1990. Oral itraconazole and topical miconazole with debridement for *Acanthamoeba* keratitis. Am. J. Ophthalmol. **109**:121–126.
12. **Jones, D. B., G. S. Visvesvara, and N. M. Robinson.** 1975. *Acanthamoeba polyphaga* keratitis and *Acanthamoeba* uveitis associated with fatal meningoencephalitis. Trans. Ophthalmol. Soc. U.K. **95**:221–232.
13. **Kilvington, S., D. F. P. Larkin, D. G. White, and J. R. Beeching.** 1990. Laboratory investigation of *Acanthamoeba* keratitis. J. Clin. Microbiol. **28**:2722–2725.
14. **Koenig, S. B., J. M. Solomon, and R. A. Hyndrick.** 1987. *Acanthamoeba* keratitis associated with gas-permeable contact lens wear. Am. J. Ophthalmol. **103**:832–833.
15. **Ma, P., G. S. Visvesvara, A. J. Martinez, F. H. Theodore, P.-M. Daggett, and T. K. Sawyer.** 1990. *Naegleria* and *Acanthamoeba* infections: review. Rev. Infect. Dis. **12**:490–513.
16. **Ma, P., E. Willaert, K. B. Juechter, and A. R. Stevens.** 1981. A case of keratitis due to *Acanthamoeba* in New York, New York, and features of 10 cases. J. Infect. Dis. **143**:662–667.
17. **MacDonald, D. L., and W. L. Maloy.** 1990. Program Abstr. 30th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 791.
18. **Maloy, W. L. (Magainin Pharmaceuticals Inc.).** 1992. Personal communication.
19. **Martinez, A. J.** 1985. Free-living amebas: natural history, prevention, diagnosis, pathology, and treatment of disease. CRC

Press, Inc., Boca Raton, Fla.
20. **Moore, M. B., and J. P. McCulley.** 1989. *Acanthamoeba* keratitis associated with contact lenses: six consecutive cases of successful management. Br. J. Ophthalmol. **73:**271–275.
21. **Moore, M. B., J. P. McCulley, and C. Newton.** 1987. *Acanthamoeba* keratitis: a growing problem in soft and hard contact lens wearers. Ophthalmology **94:**1654–1661.
22. **Nagington, J., and J. E. Richards.** 1976. Chemotherapeutic compounds and Acanthamoebae from eye infections. J. Clin. Pathol. **29:**648–651.
23. **Olson, C.** 1989. Increasing use of contact lens prompts issuing of infection-prevention guidelines. JAMA **261:**343–344.
24. **Osato, M. S., N. M. Robinson, K. R. Wilhelmus, and D. B. Jones.** 1991. In vitro evaluation of antimicrobial compounds for cysticidal activity against *Acanthamoeba*. Rev. Infect. Dis. **13**(Suppl. 5)**:**S431–S435.
25. **Schuster, F. L., and L. S. Jacob.** 1990. Program Abstr. 30th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 796.
26. **Schuster, F. L., and N. Mandel.** 1984. Phenothiazine compounds inhibit in vitro growth of pathogenic free-living amoebae. Antimicrob. Agents Chemother. **25:**109–112.
27. **Sharma, S., M. Srinivasan, and C. George.** 1990. *Acanthamoeba* keratitis in non-contact lens wearers. Arch. Ophthalmol. **108:** 676–678.
28. **Stehr-Green, J. K., T. M. Bailey, F. H. Brandt, J. H. Carr, W. W. Bond, and G. S. Visvesvara.** 1987. *Acanthamoeba* keratitis in soft contact lens wearers. A case-control study. JAMA **258:**57–60.
29. **Stevens, A. R., and E. Willaert.** 1980. Drug sensitivity and resistance of four *Acanthamoeba* species. Trans. R. Soc. Trop. Med. Hyg. **74:**806–808.
30. **Wright, P., D. Warhurst, and B. R. Jones.** 1985. *Acanthamoeba* keratitis successfully treated medically. Br. J. Ophthalmol. **69:**778–782.
31. **Yeoh, R., D. C. Warhurst, and M. G. Falcon.** 1987. *Acanthamoeba* keratitis. Br. J. Ophthalmol. **71:**500–503.
32. **Zasloff, M.** 1987. Magainins, a class of antimicrobial peptides from *Xenopus* skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc. Natl. Acad. Sci. USA **84:**5449–5453.
33. **Zasloff, M., B. Martin, and H. C. Chen.** 1988. Antimicrobial activity of synthetic magainin peptides and several analogues. Proc. Natl. Acad. Sci. USA **85:**910–913.