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NOTES

Activity of Pentamidine and Pentamidine Analogs against *Toxoplasma gondii* in Cell Cultures†

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The capabilities of pentamidine and nine pentamidine analogs to inhibit the development of *Toxoplasma gondii* were examined in vitro. Treatment of infected cultures with pentamidine and five of its analogs caused a significant ($P < 0.05$) reduction in the numbers of tachyzoites produced. Analogs of pentamidine may be useful agents in the treatment of toxoplasmosis.

Toxoplasma gondii is an ubiquitous protozoan parasite that can be an important disease-producing agent in humans and warm-blooded animals (6). In humans, *T. gondii* infections in immunocompetent patients are usually asymptomatic, whereas infections in immunocompromised patients or transplacentally infected infants can be life-threatening. Encephalitis caused by *T. gondii* has become recognized as a serious, often fatal, manifestation of the infection seen in many patients with AIDS (9, 15, 21). Toxoplasmic encephalitis (TE) is probably caused by reactivation of latent tissue cyst stages (7). Pyrimethamine alone or in combination with sulfadiazine or trisulfapyrimidines have been the standard treatments for acute toxoplasmosis and TE, although adverse reactions are common (9, 15, 17). Relapse of TE is common once treatment has been stopped. Additional compounds that do not cause adverse side effects and that have activity against *T. gondii* must be discovered and evaluated for the treatment of acute toxoplasmosis and TE.

Pentamidine and its analogs have been shown to have a broad spectrum of activity against intracellular and extracellular protozoan parasites as well as *Pneumocystis carinii* (2, 11, 12, 18–20). Because of this demonstrated effectiveness against parasitic protozoa, we examined the capabilities of pentamidine and nine of its analogs to inhibit replication of the highly pathogenic RH isolate of *T. gondii* in cell cultures.

Pentamidine and the pentamidine analogs (Table 1) used in this study were synthesized in the laboratories of Richard R. Tidwell, Department of Pathology, School of Medicine, University of North Carolina at Chapel Hill. The methods of their production and examinations for purity have been described previously (19). Pentamidine and its analogs were examined at 100 μg/ml; compounds found to be toxic at this concentration were subjected to further study at lower concentrations (Tables 2 and 3). Pyrimethamine (lot no. 3f0991) and piritrexim (lot no. 87/5089-036; Burroughs Wellcome Co., Research Triangle Park, N.C.) were used at 10 μg/ml of medium in two experiments to ensure that our assay would identify effective compounds.

African Green monkey kidney cells (Vero) (ATTC CCL 81; American Type Culture Collection, Rockville, Md.) were grown to monolayers in 25-cm^2 plastic tissue culture flasks in RPMI 1640 medium containing 10% (vol/vol) fetal bovine serum, 100 U of penicillin G per ml, 100 μg of dihydrostreptomycin (GIBCO, Grand Island, N.Y.) per ml, and 5×10^{-2} mM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.). Cell cultures were maintained in an identical medium, but the concentration of fetal bovine serum was lowered to 2% (vol/vol). Cell cultures were incubated at 37°C in a 95% air–5% CO_2 atmosphere.

Tachyzoites of the RH isolate of *T. gondii* are maintained in the laboratories of David S. Lindsay and Byron L. Blagburn, Department of Pathobiology, Auburn University, by serial passage in infected Vero cell cultures. Tachyzoites were collected for inoculation of cell cultures by first removing the cell culture medium and replacing it with fresh maintenance medium. The Vero cells were then scraped off the plastic growth surface into the medium, and the suspension was forced through a syringe equipped with a 27-gauge needle to rupture most infected Vero cells. The free tachyzoites were separated from intact cells and most cellular debris by filtration through a sterile 3-μm-pore-size polycarbonate filter (Nuclepore Corp., Pleasanton, Calif.). The numbers of tachyzoites present were determined by counting them in a hemacytometer, and the volume of inoculum was adjusted so that 1 ml contained 2×10^5 tachyzoites. For each treatment, four 25-cm^2 flasks were each inoculated with 2×10^5 tachyzoites. Two hours postinoculation (p.i.), the tachyzoite inoculum was removed and replaced with test compounds in maintenance medium. Pentamidine, pentamidine analogs, and piritrexim were solubilized in deionized water and diluted in maintenance medium, whereas pyrimethamine was solubilized in 100% ethanol. Controls received maintenance medium to which an equal amount of deionized water was added. Media were removed from the infected cells 4 to 4.5 days p.i., the volume was recorded, and the numbers of tachyzoites present were determined by counting them in a hemacytometer. The total number of tachyzoites present in each flask was determined by multiplying the volume of the medium by the numbers of tachyzoites present (mean of six counts per flask) per milliliter of

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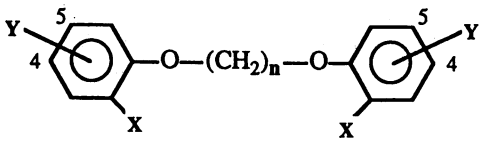
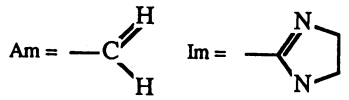
TABLE 1. Structures and names of pentamidine analogs



Compound no.	Structure component			Position Y	Compound name
	n	*X*	*Y*[a]		
1	3	$-OCH_3$	Am	4	1,3-Di(4-amindino-2-methoxyphenoxy)propane
2	3	$-H$	Im	4	1,3-Di(4-imidazolinophenoxy)propane
3	4	$-OCH_3$	Im	4	1,4-Di(4-imidazolino-2-methoxyphenoxy)butane
4	4	$-H$	Am	4	1,4-Di(4-amidinophenoxy)butane
5	3	$-OCH_3$	Im	4	1,3-Di(4-imidazolino-2-methoxyphenoxy)propane [DIMP]
6	3	$-OCH_3$	Im	5	1,3-Di(5-imidazolino-2-methoxyphenoxy)propane
7	5	$-OCH_3$	Im	4	1,5-Di(4-imidazolino-2-methoxyphenoxy)pentane
8	4	$-OCH_3$	Am	4	1,4-Di(4-amidino-2-methoxyphenoxy)butane [dimethoxybutane]
9	6	$-H$	Am	4	1,6-Di(4-amidinophenoxy)hexane [hexamidine]
10	5	$-H$	Am	4	1,5-Di(4-amidinophenoxy)pentane [pentamidine]

[a]



medium. Mean total tachyzoite production was analyzed by a nonparametric one-tailed Mann-Whitney test. Designation of significant differences in all experiments was based on a cutoff of $P < 0.05$. The percent reduction in tachyzoites was calculated by subtracting the mean treated values from the mean control value, dividing this numerator by the mean control value, and multiplying the product by 100. Eight experiments were conducted (Tables 2 and 3).

Pentamidine and compounds 3, 6, 7, 8, and 9 reduced the numbers of *T. gondii* RH tachyzoites in cell cultures (Tables 2 and 3). Because compounds 3, 7, and 8 appeared to be highly effective at 100 μg/ml, additional doses were examined (Table 3). Compounds 1, 2, 4, and 5 had no significant effects on tachyzoite production. Pentamidine and compound 9 were toxic at 100 and 50 μg/ml, and compound 4 was toxic at 100 μg/ml for Vero cell cultures. These compounds were considered toxic because many of the Vero cells became rounded and detached from the plastic growth surface by 1 to 3 days p.i. in these flasks, whereas Vero cells in flasks infected with *T. gondii* and not treated did not exhibit these cytopathic changes. When pentamidine was evaluated at 25 and 10 μg/ml (Table 3), significant effects on

TABLE 2. Activities of pentamidine analogs against *T. gondii* in Vero cell cultures[a]

Expt no.	Compound[b]	Total no. of tachyzoites/ flask (mean ± SD [10^4])	% Reduction[c]
1	Control	597 ± 166	NA
	1 (100)	1,088 ± 408	0
	2 (100)	950 ± 287	0
	3 (100)	45 ± 11	92.5*
2	Control	672 ± 173	NA
	5 (100)	1,325 ± 633	0
3	Control	1,367 ± 141	NA
	4 (50)	1,065 ± 233	22.1
	6 (100)	649 ± 304	52.5*
4	Control	479 ± 120	NA
	7 (100)	28 ± 13	94.2*
	8 (100)	102 ± 51	78.7*
	Pyrimethamine (10)	3 ± 3	99.4*

[a] Pentamidine and compound 9 were toxic at 100 and 50 μg/ml, and compound 4 was toxic at 100 μg/ml.

[b] Control cultures received no drug treatment and treated cultures received treatment at the concentration (in micrograms per milliliter) given in parentheses.

[c] NA, not applicable; negative percent reductions are expressed as 0; *, significant reduction in the number of tachyzoites per flask compared with that in control flasks ($P < 0.05$).

TABLE 3. Dosage evaluations of pentamidine and compounds 3, 7, 8, and 9 against *T. gondii* in Vero cell cultures

Expt no. (compound)	Dose (μg/ml)[a]	Total no. of tachyzoites/flask (mean ± SD [10^4])	% Reduction[b]
5 (compound 3)	Control	1,093 ± 108	NA
	10	859 ± 268	21.4
	50	176 ± 97	83.9*
6 (pentamidine)	Control	579 ± 116	NA
	10	230 ± 56	60.3*
	25	182 ± 95	68.6*
7	Control	316 ± 157	NA
Compound 7	10	423 ± 85	0
	50	62 ± 23	80.4*
Compound 8	10	809 ± 234	0
	50	132 ± 66	58.2
8	Control	506 ± 125	NA
Compound 9	10	92 ± 62	81.8*
Piritrexim	10	1 ± 1	99.8*

[a] Control cultures received no drug treatment.

[b] NA, not applicable; negative percent reductions are expressed as 0; *, significant reduction in the number of tachyzoites per flask compared with that in control flasks ($P < 0.05$).

tachyzoite replication were observed. Compound 9 was effective when it was examined at 10 μg/ml (Table 3). Pyrimethamine and piritrexim both significantly inhibited tachyzoite replication in our assay at a concentration of 10 μg/ml (Tables 2 and 3).

Many antimicrobial agents have been evaluated for their effectiveness against *T. gondii*; these include dihydrofolate reductase inhibitors, macrolide antibiotics, polyether ionophorous antibiotics, sulfonamides, 1,2,4-trioxanes, hydroxynaphthoquinones, and purine analogs (1, 3–5, 8, 10, 13, 14, 16, 17). However, the present study appears to be the first to evaluate the effectiveness of pentamidine and some of its analogs against *T. gondii*.

The present study demonstrated that pentamidine and five of its analogs are effective in inhibiting replication of the RH isolate of *T. gondii* in cell cultures and suggests that other analogs of pentamidine may also be effective against the parasite. Future studies should focus on evaluation of these additional analogs. Additionally, studies must be conducted to determine whether synergistic effects are observed when these compounds are administered with other effective compounds.

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