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Albendazole Inhibits *Pneumocystis carinii* Proliferation in Inoculated Immunosuppressed Mice

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Albendazole, a benzimidazole derivative widely used for treating helminth infections, was successfully used to treat and prevent development of *Pneumocystis carinii* pneumonia in transtracheally inoculated immunosuppressed mice. For treatment, 3 weeks postinoculation, albendazole at 300 and 600 mg/kg of body weight per day was administered in food for 3 weeks. For prophylaxis, albendazole was begun on the same day as inoculation at 300 mg/kg/day for 7 days, and then the dose was reduced to 150 mg/kg/day for 35 additional days. With these regimens, albendazole was effective both for treatment and prophylaxis. Both dexamethasone-immunosuppressed and L3T4$^+$ monoclonal antibody-immunosuppressed mouse models were used, and albendazole inhibited *P. carinii* infection in both.

Pneumocystis carinii pneumonia is the most common opportunistic infection in AIDS patients in North America, occurring in up to 85% of those with AIDS (5). Adverse reactions to treatments and prophylaxis with trimethoprim-sulfamethoxazole and the toxicity of pentamidine have prompted a search for other drug regimens. Compounds other than anti-folate pathway agents may avoid some reactions, and drugs with new mechanisms of action may enhance capabilities for treating and preventing *P. carinii* pneumonia.

Benzimidazoles are compounds that disrupt microtubule function by blocking polymerization of the α- and β-tubulin subunits. Various benzimidazoles have been used in agriculture as antifungal agents and in veterinary and human medicine for treating intestinal helminth infections (6, 7, 15). The derivative albendazole has proven useful for treating systemic infections as well and is now an accepted therapeutic agent for cysticercosis (19) and echinococcosis (14, 16, 18). Recently, albendazole has also been shown to be effective in the treatment of infections caused by the protozoans *Giardia lamblia* (11, 17) and *Enterocytozoon bieneusi* (4, 10). Albendazole was also active in vitro against *G. lamblia* (13). Albendazole was evaluated for effect on *P. carinii* in vitro with short-term-cultured *P. carinii* from rats and was found to be inhibitory (2). This activity prompted us to evaluate albendazole in animal models of *Pneumocystis* pneumonia.

MATERIALS AND METHODS

Inoculation, treatment, and evaluation of mice. Female BALB/c mice 6 to 8 weeks of age (colony 202; Harlan Sprague Dawley, Indianapolis, Ind.) were transtracheally inoculated with *P. carinii* from infected mouse lung as reported previously (3). Briefly, mice were immunosuppressed throughout the study with dexamethasone at 1.2 mg/kg of body weight per day administered in drinking water or with monoclonal antibody from clone GK1.5 directed to L3T4$^+$ cells (9) at 0.2 mg per dose given intraperitoneally twice a week. One treatment study was with the dexamethasone-immunosuppressed mouse model only, and the other used this model and the L3T4$^+$-immunosuppressed model. After 11 (dexamethasone) or 14 (antibody) days of immunosuppression, mice were transtracheally inoculated with about 10^6 trophozoites and cysts in 50 μl of infected mouse lung homogenate. Mice were anesthetized with ketamine cocktail (ketamine hydrochloride, 80 mg/ml; acepromazine, 1.78 mg/ml; atropine, 0.38 mg/ml), a small midline incision was made, the trachea was exposed by blunt dissection, the inoculum was injected directly into the trachea, and the wound was closed with a clip. Mice were randomized to treatment groups 3 weeks after inoculation and treated for 3 weeks or were started on prophylaxis the same day they were inoculated. Mice in groups of 10 were assigned to treatment with albendazole at 600 or 300 mg/kg/day for 3 weeks, prophylaxis with albendazole for 5 days at 300 mg/kg/day and then for 35 days at 150 mg/kg/day (given in peanut butter and rodent chow), 50 mg of trimethoprim per kg/day plus 250 mg of sulfamethoxazole per kg/day (given in drinking water), or were untreated. For evaluation, mice were anesthetized with ketamine cocktail and exsanguinated by cardiac puncture, lungs were removed and weighed, and samples were used for impression smears that were Giemsa and methenamine silver nitrate stained and evaluated microscopically as unknowns by two individuals as described previously (1) or were evaluated by enzyme-linked immunosorbent assay (ELISA) (12). Microscopic scores were assigned according to the following scheme: 5+, >100 organisms per ×1,000 microscopic field; 4+, 11 to 100 organisms per ×1,000 microscopic field; 3+, 1 to 10 organisms per ×1,000 microscopic field; 2+, 2 to 9 organisms in 10 ×1,000 microscopic fields; 1, 1 organism in 10 to 50 microscopic fields; and 0, no organisms in 50 microscopic fields. Mean scores and standard errors were calculated.

ELISA evaluation. ELISA was performed as described previously (12). Briefly, a portion of each lung was cut, weighed, and ground in phosphate-buffered saline (PBS) to yield 10 mg of tissue per ml of solution. One-milliliter samples of each were pelleted and washed twice with PBS, and pellets were covered with 200-μl solutions of 1 M urea–1 mg of dithiothreitol per ml. Antigens were vortexed, and PBS was

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TABLE 1. Treatment study results

Treatment	Dose (mg/kg/day)	Immunosuppressant	Infectivity score (no. infected/total) with:		ELISA absorbance
			Giemsa stain[a]	Silver stain	
Study 1					
Albendazole	600	Dexamethasone	2.4 ± 0.4[b] (6/7)	2.5 ± 0.3 (7/7)	0.203 ± 0.015
Albendazole	300	Dexamethasone	2.7 ± 0.5[c] (7/8)	2.6 ± 0.4 (7/8)	0.229 ± 0.033
TMP-SMX[d]	50 (TMP), 250 (SMX)	Dexamethasone	0.1 ± 0.1[e] (1/10)	0 (0/10)	0.121 ± 0.001
None (control)		Dexamethasone	3.8 ± 0.5 (8/9)	3.3 ± 0.4 (8/9)	0.458 ± 0.044
Study 2					
Albendazole	600	L3T4$^+$	0.9 ± 0.3[e,f] (9/10)	1.2 ± 0.2 (10/10)	
Albendazole	600	Dexamethasone	2.2 ± 0.3[g] (9/9)		
TMP-SMX	50 (TMP), 250 (SMX)	L3T4$^+$	0.1 ± 0.1[e] (3/10)	0.1 ± 0.1 (3/10)	
TMP-SMX	50 (TMP), 250 (SMX)	Dexamethasone	0.0[e] (0/10)	0.1 ± 0.1 (1/10)	
None (control)		L3T4$^+$	4.3 ± 0.2 (10/10)	3.1 ± 0.1 (10/10)	
None (control)		Dexamethasone	3.7 ± 0.3 (10/10)	3.1 ± 0.2 (10/10)	

[a] *P* values reflect comparison of the experimental set with its own control. *P* values of <0.05 are considered significant.
[b] $P = 0.0205$.
[c] $P = 0.0148$.
[d] TMP-SMX, trimethoprim-sulfamethoxazole.
[e] $P < 0.0001$.
[f] $P = 0.0033$ versus albendazole-dexamethasone.
[g] $P = 0.0057$.

added to bring volumes to 1 ml. In 96-well plates, 100-μl samples were incubated, washed, blocked for nonspecific binding with 3% bovine serum albumin, washed, and reacted with polyclonal rat convalescent-phase antisera diluted 1:500 in PBS. Goat anti-rat immunoglobulin G conjugated with alkaline phosphatase was added, and after incubation and washing, enzyme was developed in substrate (1 mg of *p*-nitrophenyl phosphate per ml in substrate buffer [$MgCl_2$ and diethanolamine]) at 35°C and optical density was read at 405 nm on a Molecular Devices ELISA reader.

Serum and tissue albendazole assays. Albendazole was supplied by SmithKline Beecham Corporation (West Chester, Pa.). Mebendazole was supplied by Janssen Biotech (Flanders, N.J.), and Plasmanate, a plasma protein fraction prepared from pooled human plasma, was obtained from Miles, Inc. (Etobicoke, Ontario, Canada).

Albendazole assay. Stock standards of albendazole (1 mg/ml) and mebendazole (3 μg/ml, internal standard) were prepared in methanol-dimethyl sulfoxide (90:10). The stock standard of albendazole was diluted in methanol and added to drug-free Plasmanate or tissue homogenate supernatant (see below) to give concentrations ranging from 100 ng/ml to 5,000 ng/ml. Serum was obtained from clotted whole blood by centrifugation at 1,500 × *g* for 15 min. Lung tissue was homogenized in 5 volumes of 0.1 M Na_2HPO_4 (pH 6.0) and centrifuged at 1,500 × *g* for 15 min. One-half milliliter of serum or 1.0 ml of tissue homogenate supernatant was combined and mixed with 100 μl of internal standard and 1.0 ml of 0.1 M K_2CO_3. Albendazole and internal standard were extracted twice with 4 ml of CH_2Cl_2 by mixing for 10 min on an Eberbach shaker followed by centrifugation at 1,500 × *g* for 10 min. The pooled extracts were evaporated to dryness under a stream of nitrogen gas. The residue was reconstituted with 100 μl of methanol, and 10 μl was injected onto a high-pressure liquid chromatograph (HPLC). The HPLC was fitted with a Beckman Ultrasphere C_8 column (4.6 mm by 15 cm by 5 μm). The mobile phase was 70 mM monochloracetic acid, methanol, and acetonitrile (55:27:18). The flow rate was 1.2 ml/min. The column eluate was monitored at 290 nm with 0.01 absorbance units, full scale. Chromatograms were recorded on a Hewlett-Packard 3396A integrator, and the ratios of albendazole to internal standard peak heights were plotted versus albendazole concentration. Results for serum were expressed in nanograms per milliliter. Tissue albendazole levels, normalized for wet weight, were expressed in nanograms per gram.

Statistical evaluations. Evaluation of results was done with the nonparametic Mann-Whitney test.

RESULTS

Treatment of dexamethasone-immunosuppressed mice with albendazole at both 300 and 600 mg/kg/day decreased *P. carinii* infection by more than 90% compared with that in untreated controls. Infectivity scores for treated mice immunosuppressed with dexamethasone are shown in Table 1. In the second treatment study, mice immunosuppressed with dexamethasone or L3T4$^+$ antibody and treated with albendazole had 90% or greater reduction of *P. carinii* compared with control mice. Scores for the treated mice immunosuppressed with dexamethasone were comparable to the infectivity scores of the first treatment study (Table 1). Mice immunosuppressed with L3T4$^+$ antibody and treated with albendazole had mean (± standard error) scores of 0.9 ± 0.3, in contrast to the dexamethasone-immunosuppressed mice, who had Giemsa scores of 2.4 ± 0.5 for the first study and 2.2 ± 0.3 for the second. Untreated mice in all immunosuppressive groups were heavily infected.

Prophylaxis with albendazole decreased development of disease. Infectivity scores for mice given prophylaxis with albendazole are shown in Table 2. In this study, one mouse had a score of 4.0 for Giemsa stain evaluation, 3.5 for silver stain evaluation, and 0.119 for ELISA, all higher than the scores of the rest of the mice. The group scores were 1.8 ± 0.6 for Giemsa stain, 1.4 ± 0.4 for silver stain, and 0.092 ± 0.005 for ELISA, including the results for this mouse. Serum and tissue drug levels in this mouse were lower than the levels for the other mice. Serum and tissue drug levels are shown in Table 3. Each mouse group was housed together in a cage, and it is possible that the mouse with the low albendazole levels (and higher infectivity score) was less competitive for access to the drug-treated food. The mice preferred the peanut butter pellets to regular rodent chow and actively sought them.

TABLE 2. Prophylaxis study results

Treatment	Dose (mg/kg/day)	Infectivity score (no. infected/total) with:		ELISA absorbance
		Giemsa stain[b]	Silver stain	
Albendazole[a]	300 (5 days), 150 (35 days)	1.9 ± 0.4 (4/6)[c]	1.4 ± 0.4 (5/6)	0.092 ± 0.005
Trimethoprim-sulfamethoxazole	50 (trimethoprim), 250 (sulfamethoxazole)	0.1 ± 0.1 (2/10)[d]	0.0 (0/10)	0.082 ± 0.001
None (control)		4.9 ± 0.1 (10/10)	3.9 ± 0.1 (10/10)	0.221 ± 0.016

[a] One mouse (mouse 2) had infectivity scores of 4.0 and 3.5 with Giemsa and silver stains, respectively.
[b] P values reflect comparison of the experimental set with its own control.
[c] $P = 0.0005$ (extremely significant).
[d] $P < 0.0001$ (extremely significant).

DISCUSSION

The decreased infectivity scores show the effectiveness of albendazole in these mouse models of *P. carinii* pneumonia both as treatment and as prophylaxis. Albendazole was less effective than trimethoprim-sulfamethoxazole, but the doses of trimethoprim-sulfamethoxazole used in these studies are at least 10 times the minimally effective dose (20). Doses of albendazole used in these mouse studies proved to be toxic in rats, and we learned from V. J. Theodorides of SmithKline Beecham Animal Health (19a) that albendazole produced hematological dyscrasias and liver lesions in rats and that treated rats usually died of fulminating bacterial infections. The rat model was therefore inappropriate for testing albendazole. Transtracheally inoculated mice have been used to test other drugs shown to be effective against *P. carinii* in humans and rats (3) with the same predictability as the rat model. The mouse model should be appropriate for identifying potentially useful agents for human trials. Humans tolerate albendazole well. Albendazole administered orally is reasonably well absorbed and then metabolized into sulfoxide and sulfone derivatives. Although these metabolites appear to have antihelminthic activity, their activity against *P. carinii* is unknown; only the parent compound was tested in vitro (2).

Albendazole at 15 mg/kg/day is used for treatment of echinococcosis, cysticercosis, and other systemic infections. At 10 to 14 mg/kg/day, levels in lung of 700 to 800 ng/g have been reported (18). Stable levels in blood of 600 to 1,000 ng/ml have been achieved with these doses (18). Albendazole has been used for treatment of microsporidial infections, with no significant adverse effects associated with the treatment (10). The dose for *P. carinii* might be greater than the appropriate doses for *E. bieneusi* (10), but doses for treatment of human *P. carinii* pneumonia will have to be established. The effectiveness of albendazole at 1.0 μg/ml in the short-term culture system suggests that effective drug concentrations in the lung could be achieved (2).

Albendazole appeared to be more effective in the antibody-immunosuppressed mouse model than in the dexamethasone-immunosuppressed mouse model, although the numbers of animals were small and some dexamethasone-treated mice died. The L3T4$^+$ antibody described by Dialynis et al. (9) is directed to lymphocytes comparable to the CD4 helper-inducer cells of humans. This model is more analogous to the patient infected with the AIDS virus with depleted CD4 cells than is the model of the dexamethasone-immunosuppressed mouse with depletion of all leukocyte classes and may be a better model for evaluation of drugs to be used in patients with AIDS (1).

Albendazole should be considered for clinical trials and may be an agent for treatment and prevention of several infections that cause morbidity and mortality in patients with AIDS. In addition to *Pneumocystis* pneumonia, microsporidial infections have responded favorably to albendazole (4, 10) and *Cryptococcus neoformans* is susceptible to this and other benzimidazoles in vitro (8).

TABLE 3. Tissue and serum albendazole levels

Mouse	Level of albendazole in:	
	Tissue (μg/g)	Serum (ng/ml)
1	3.35	28.9
2	0.84	Below detectable levels
3	4.94	27.9
4	4.66	17.7
5	3.23	16.3
6	3.85	29.3

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REFERENCES

1. **Bartlett, M. S., W. L. Current, A. Orazi, N. L. Bauer, R. S. Neiman, S. F. Queener, and J. W. Smith.** Comparison of corticosteroid- and L3T4$^+$ antibody-immunosuppressed mouse models of *Pneumocystis carinii* for evaluation of drugs and leukocytes. Clin. Diagn. Lab. Immunol., in press.
2. **Bartlett, M. S., T. D. Edlind, M. M. Durkin, M. M. Shaw, S. F. Queener, and J. W. Smith.** 1992. Antimicrotubule benzimidazoles inhibit in vitro growth of *Pneumocystis carinii*. Antimicrob. Agents Chemother. **36**:779–782.
3. **Bartlett, M. S., S. F. Queener, M. M. Durkin, M. M. Shaw, and J. W. Smith.** 1992. Inoculated mouse model of *Pneumocystis carinii* infection. Diagn. Microbiol. Infect. Dis. **15**:129–134.
4. **Blanshard, C., D. S. Ellis, D. G. Tovey, S. Dowell, and B. G. Gazzard.** 1992. Treatment of intestinal microsporidiosis with albendazole in patients with AIDS. AIDS **6**:311–313.
5. **Centers for Disease Control.** 1989. AIDS weekly surveillance report. 30 January, p. 1–5. Centers for Disease Control, Atlanta.
6. **Chanthavanich, P., P. Nontasut, V. Prarinyanuparp, and S. Sa-Nquankist.** 1989. Repeated doses of albendazole against strongyloidiasis in Thai children. Southeast Asian J. Trop. Med. Public Health **20**:221–226.
7. **Cline, B. L., M. D. Little, R. K. Bartholomew, and N. A. Halsey.** 1984. Larvicidal activity of albendazole against *Necator americanus* in human volunteers. Am. J. Trop. Med. Hyg. **33**:387–394.
8. **Cruz, M. C., M. S. Bartlett, and T. D. Edlind.** 1994. In vitro susceptibility of the opportunistic fungus *Cryptococcus neoformans* to anthelmintic benzimidazoles. Antimicrob. Agents Chemother. **38**:378–380.
9. **Dialynas, D. P., D. B. Wilde, P. Marrack, A. Pierres, K. A. Wall, H. Havran, G. Otten, M. R. Loken, M. Pierres, F. Kappler, and F. W. Fitch.** 1983. Characterization of the murine antigenic determinant,

designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen reactivity. Immunol. Rev. **74**:29–56.

10. **Dieterich, D. T., E. A. Lew, D. P. Kotler, M. A. Poles, and J. M. Orenstein.** 1994. Treatment with albendazole for intestinal disease due to *Enterocytozoon bieneusi* in patients with AIDS. J. Infect. Dis. **169**:178–183.
11. **Di Rosa, S., M. Affronti, and R. Malta.** 1989. The treatment of giardiasis with albendazole. J. Chemother. **1**(Suppl. 4):948.
12. **Durkin, M. M., M. S. Bartlett, S. F. Queener, M. M. Shaw, C.-H. Lee, and J. W. Smith.** 1992. An enzyme-linked immunosorbent assay for enumeration of *Pneumocystis carinii* in vitro and in vivo. J. Clin. Microbiol. **30**:3258–3262.
13. **Edlind, T. D., T. L. Hang, and P. R. Chakraborty.** 1990. Activity of the anthelmintic benzimidazoles against *Giardia lamblia* in vitro. J. Infect. Dis. **162**:1408–1411.
14. **Horton, R. J.** 1989. Chemotherapy of *Echinococcus* infection in man with albendazole. Trans. R. Soc. Trop. Med. Hyg. **83**:97–102.
15. **Maisonneuve, H., and J. F. Rossignol.** 1985. Ovicidal effects of albendazole in human ascariasis, ancylostomiasis and trichuriasis. Ann. Trop. Med. Parasitol. **79**:79–82.
16. **Morris, D. L., P. W. Dykes, B. Dickson, S. E. Marriner, J. A. Bogan, and F. G. O. Burrows.** 1983. Albendazole in hydatid disease. Br. Med. J. **286**:103–104.
17. **Reynoldson, J. A., R. C. A. Thompson, and R. J. Horton.** 1992. Albendazole as a future antigiardial agent. Parasitol. Today **8**:412–414.
18. **Saimot, A. G., A. C. Cremieux, J. M. Hay, A. Meulemans, M. D. Giovanangeli, B. Delaitre, and J. P. Coulaud.** 1983. Albendazole as a potential treatment for human hydatidosis. Lancet **ii**:652–656.
19. **Sotelo, J., O. H. del Brutto, P. Penagos, F. Escobedo, B. Torres, J. Rodriguez-Carbajal, and F. Rubio-Donnadieu.** 1990. Comparison of therapeutic regimen of anticysticercal drugs for parenchymal brain cysticercosis. J. Neurol. **237**:69–72.

19a. **Theodorides, V. J. (SmithKline Beecham Animal Health).** Personal communication.

20. **Walzer, P. D., J. Foy, P. Steele, C. K. Kim, M. White, R. S. Klein, B. A. Otter, and C. Allegra.** 1992. Activities of antifolate, antiviral, and other drugs in an immunosuppressed rat model of *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **36**:1935–1942.