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Susceptibility of *Pneumocystis carinii* to Artemisinin In Vitro

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The susceptibility of *Pneumocystis carinii* to artemisinin (qinghaosu) was determined in short-term primary culture. In untreated cultures, trophozoites increased an average of fivefold over 4 days. Inhibition of parasite growth in cultures treated with artemisinin at concentrations as low as 0.5 μM was seen. In contrast, artemisinin concentrations up to 100 μM had no effect on feeder layer cells.

Pneumocystis carinii is an opportunistic pathogen which causes pneumonia in AIDS patients and other immunocompromised patients; it is estimated that up to 150,000 cases of *P. carinii* pneumonia will occur in the United States over the next 3 years (8). Patients with *P. carinii* pneumonia are usually treated with pentamidine or antifolates but frequently develop adverse reactions to these drugs. In AIDS patients with *P. carinii* pneumonia, side effects occur in more than 60% of treated patients (6). New, less toxic, and more effective therapeutic agents are needed.

Artemisinin (qinghaosu) is a promising new antimalarial agent derived from an ancient Chinese herbal remedy. Artemisinin and its derivatives, particularly artemether, have shown remarkable efficacy and are now undergoing clinical trials against drug-resistant malaria (2, 4, 9, 11, 15). In addition, artemisinin and artemether have recently been shown to be active against other parasitic organisms, including *Toxoplasma gondii* and *Schistosoma mansoni* (12, 13). We report here that artemisinin is also active against *P. carinii* in vitro.

Growth, isolation, and cultivation of organisms. Male Sprague-Dawley rats (150 to 200 g; Taconic Farms, Germantown, N.Y.) were fed ad libitum and administered tetracycline in their drinking water (1 g/liter). Subcutaneous injections of cortisone acetate (25 mg) were given twice per week. After 4 to 6 weeks of injection, those rats which showed signs of sluggishness, rapid breathing, weight loss, and hair loss were utilized in this study.

The rats were sacrificed and their lungs were then quickly removed and kept at 4°C. Impression smears were made from the lungs and stained with toluidine blue (7) and Gram stain. The experiment was continued if cysts were present but if no fungi or bacteria were evident. Subsequent inoculation of lung homogenates on blood-agar plates confirmed the absence of contaminants. The lungs were homogenized by repeated passage through a 60-gauge wire screen. The resulting suspension was centrifuged at 100 × g for 5 min. The supernatant was removed and used for the culture.

Trophozoites were identified by Giemsa stain. Two microliters of the lung supernatant was placed on a 1-cm² square etched on a glass slide with a diamond stylus. The slides were air dried, fixed in methanol for 3 min, and stained with Giemsa stain (4%, modified azure blend type; Harleco, Gibbstown, N.J.) for 40 min. Trophozoites were counted by a double-blind method by light microscopy.

P. carinii was cultivated by a modification of previously

published methods (1, 3). Twenty-four-well plates were seeded with 5 × 10⁵ cells of epithelioid mink lung cell line Mv 1 Lu (American Type Culture Collection, Rockville, Md.). Each well was incubated for 36 h or until the cells were >90% confluent in Eagle's minimal essential medium (Sigma Chemical Co., St. Louis, Mo.) containing 10% heat-inactivated fetal calf serum (Grand Island Biological Company, Grand Island, N.Y.), penicillin (100 U/ml), and streptomycin (100 μg/ml). Each well was then inoculated with 2 × 10⁵ trophozoites from supernatants of infected-lung homogenates. The cultures were incubated in 5% O_2 and 10% CO_2 at 37°C (3). For counting, the organisms were dislodged with a transfer pipette and then counted as described above. For each experiment, the cells from an entire well were harvested and counted each day for 4 days.

Effect of artemisinin on *P. carinii* and feeder layer cells in culture. Artemisinin was obtained from the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases, Geneva, Switzerland, and stored as an ethanolic solution at −70°C. Artemisinin was added at the following concentrations to cultures immediately after inoculation: 100, 50, 5, 0.5, and 0.1 μM. The final ethanol concentration was never greater than 0.3%. A pentamidine concentration of 15 μM was used as a positive control.

P. carinii trophozoites increased in number for 4 days by an average of 5.3-fold (Fig. 1). The inhibitory effect of pentamidine was not seen on day 1 of culture (<26%) but was evident on days 2 and 3 of culture, when inhibitions by 63 and 79%, respectively, were seen. In contrast, the effect of 100 μM artemisinin was evident after 1 day (Fig. 2), with growth inhibited by 63, 76, and 78% on days 1, 2, and 3, respectively.

Artemisinin at concentrations of 50 and 5 μM also completely inhibited the growth of *P. carinii* for 4 days, without affecting the feeder layer cells (Fig. 3). No effect at an artemisinin concentration of 0.1 μM was observed, although artemisinin at 0.5 μM had an intermediate effect.

As controls, feeder layer cells were incubated for 4 days in the presence of 100, 50, 10, and 1 μM artemisinin but in the absence of parasites, under conditions identical to those used for parasite culture. No effect was observable by phase-contrast microscopy. Feeder layer cells from the 100 μM artemisinin control were also dislodged from the wells by trypsinization and found viable by trypan blue exclusion (>95%). Furthermore, 0.5% ethanol had no effect either on the feeder layer cells or on parasite growth over 4 days (data not shown).

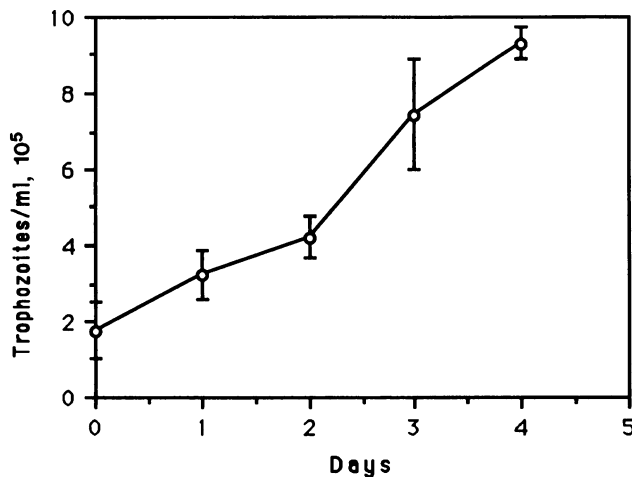**Conclusion.** Artemisinin, a potent antimalarial agent, in-

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FIG. 1. Growth of *P. carinii* in culture. Each point is the average of four experiments ± standard deviation.

hibits the growth of *P. carinii* in culture at concentrations as low as 0.5 μM. In contrast, artemisinin has no effect on Mv 1 Lu feeder layer cells at a concentration of 100 μM, indicating that the drug is selectively toxic to the parasite. An artemisinin concentration of 0.5 μM caused a 55% inhibition of growth. This is similar to the concentration which inhibits *T. gondii*, 0.3 μM (12), but is considerably higher than the concentration which inhibits plasmodium growth, 20 nM (10). Nevertheless, concentrations which inhibit growth of *P. carinii* in vitro are close to the levels achieved in patients treated with artemisinin. For example, the peak concentration in plasma is 0.4 μM in patients 8 to 12 h after administration of artemisinin suppositories and is 3.5 μM in patients after intramuscular artemether administration (14).

In this study, we used a short-term in vitro culture system to assess the effects of artemisinin on *P. carinii*. Others have reported increases in the number of trophozoites for as long as 10 days, accompanied by a 2- to 10-fold increase in cell number (5). Short-term cultures have also been used to demonstrate the antipneumocystis activities of trimetho-
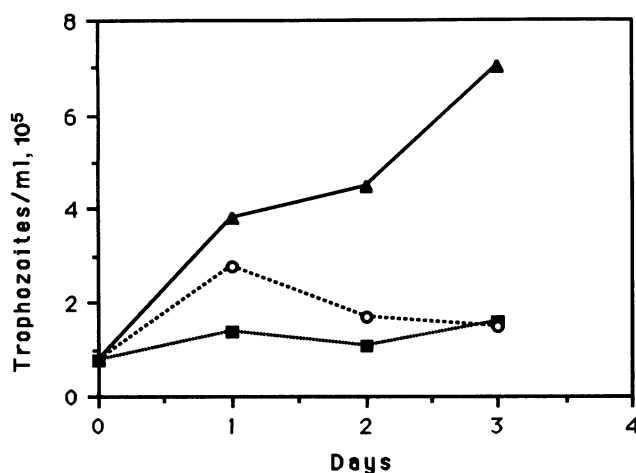

FIG. 2. Growth of *P. carinii* in culture in the presence of 100 μM artemisinin (■), 15 μM pentamidine (○), or no addition (▲).
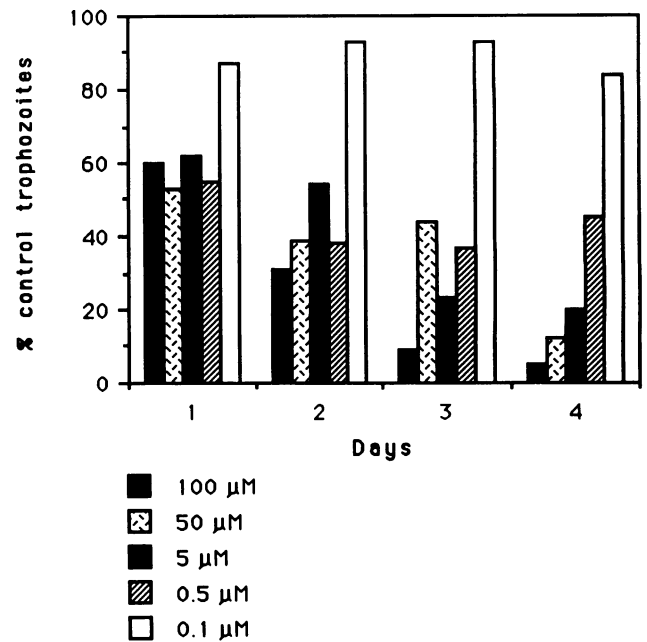

FIG. 3. Inhibition of *P. carinii* growth in culture by various concentrations of artemisinin.

prim-sulfamethoxazole, pentamidine, dapsone, primaquine, trimetrexate, and other drugs (5).

Artemisinin and its derivatives represent a promising new class of antiparasitic drugs which act as free-radical generators (10). They are currently being used in the treatment of malaria in China (4, 9, 15), Burma (11), and Vietnam (2). The results presented here suggest that they are potentially useful in the treatment of *P. carinii* pneumonia.

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