Antimalarial Activity of Actinomycin D and Cyclophosphamide[1] (34172)

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(Introduced by E. R. Fisher)

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In spite of the years of efforts since the synthesis of pamaquine in 1924, quinacrine in 1930, and chloroquine in 1934 (1), there is still no single synthetic drug known to eradicate both the blood and tissue stages of the different types of malaria parasites encountered in man (2). The search for newer and more active antimalarial agents has been further intensified by the recent appearance of chloroquine-resistant strains (2–4). Because most drugs currently being designed or tested against malaria supposedly act by interfering with the parasite's replicatory functions, and since the trinity of host–drug–parasite is a similar obstacle in the area of cancer chemotherapy, we postulate that drugs active against neoplasms might present some degree of toxic activity against plasmodia parasites, and perhaps even, in certain cases, vice versa. In testing this supposition, the antimalarial effects of actinomycin D and cyclophosphamide, two of the most active antineoplastic agents now in clinical use, are first considered. This report presents evidence that they are indeed effective antimalarial agents in an *in vivo* murine system.

Materials and Methods. Antimalarial activity was evaluated by determining percentage reduction of parasitemia in mice inoculated with malaria plasmodia. The strain of malaria employed with *Plasmodium vinckei.* This was described by Rodhain in 1952 from the African tree-rat, *Thamnomys surdaster*, which was also the host in which *P. berghei* had been found [(1), p. 111]. It is smaller than *P. berghei,* usually producing about eight merozoites per segmenter in the mouse, and, unlike *P. berghei*, does not shown any predilection for reticulocytes. Its natural vector is believed to be *Anopheles dureni* (5), the same source as for *P. berghei.* It is not resolved whether *P. vinckei* is a variant of *P. berghei*; nevertheless, Rodhain has shown that is is now antigenically distinct, since no cross-immunity exists between these two plasmodia species [(1), p. 111].

P. vinckei is highly virulent in mice of the NMRI strain and has proved to be a convenient system for evaluating schizonticidal activity even after only a single drug application on the day following infection of the host. We have found that, depending upon the number of parasites injected, almost 100% of the animals expire between 4–8 days post infection (W. Kretschmar and E. Fink, in preparation). Since there is a dependent relationship between survival time and degree of parasitism, the former parameter can be estimated by determining reduction in parasitemia percentage following short-term chemotherapy.

Female NMRI mice weighing 18–20 g, maintained on Altromin-R laboratory chow and water *ad libitum*, were inoculated intraperitoneally with 1×10^7 parasitized erythrocytes derived from 3 to 4-day-old parasitized donors, and drug therapy instituted on the same and/or 1 day later. Blood smears from treated and control mice were made on the second day post infection, or 1 day after therapy; at this time, untreated mice have 4–6% of their erythrocytes parasitized. The antimalarial activity of the drug tested is

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TABLE I. Suppressive Activity of Cyclophosphamide and Actinomycin D against *Plasmodium vinckei* in Mice Infected with 1×10^7 Parasitized Erythrocytes.

		Parasitemia 2 days post infection (no. of parasit. RBC/micr. field)					
		Treated		Controls			
Drug	Dose schedule (mg/kg) (therapy days)[a]	No. of mice	($\bar{x} \pm SE\bar{x}$)	No. of mice	($\bar{x} \pm SE\bar{x}$)	Reduction of parasitemia (%)	Significance p
Cyclophosphamide	1×100 (0,1)	8	10.1 ± 2.0	8	39.8 ± 3.1	75	<0.001
	1×20 (0,1)	8	25.3 ± 3.9	8	39.8 ± 3.1	37	<0.02
Actinomycin D	2×0.01 (1)	8	10.7 ± 1.3	8	23.1 ± 2.7	53	<0.001
	1×0.01 (1)	8	9.6 ± 2.1	8	23.1 ± 2.7	58	<0.005
Chloroquine	1×1.0 (0)	13	25.0 ± 2.4	16	41.4 ± 4.5	40	<0.005

[a] Day 0 = day of infection; Day 1 = 1 day post infection.

expressed in percentage suppression of parasitemia, based on the control values, at this time (day 2). Percentage parasitemia was determined by counting 10 fields/slide. Each field contained approximately 600 red blood cells.

Actinomycin D (Serva) and cyclophosphamide (Endoxan, Cytoxan) were obtained in their commercial forms and so diluted in physiological saline as to provide 0.1 ml/10 g mouse per ip injection. For purposes of comparison, a standard antimalarial agent, chloroquine (Resochin), was included in these experiments.

Results. The results obtained with the three drugs tested are summarized in Table I. Cyclophosphamide administered in doses of 20 and 100 mg/kg on days 0 and 1 yielded a reduction of parasitemia of 37 and 75%, respectively. A dose of 10 μg/kg of actinomycin D applied once and twice on day 1 post-infection resulted in about the same level of antimalarial activity (53–58%), which is particularly striking because of the extremely small amount of drug administered (the single dose LD_{10} for actinomycin D in mice ranges from about 150–250 μg/kg of body wt). At the dosages employed, both anticancer agents revealed higher antimalarial activity in this system than chloroquine. Based on Student's t test, these inhibition percentages are significant.

Discussion. Both cancerostatic agents shown here to possess antimalarial activity in a murine *P. vinckei* system supposedly produce cytotoxic effects by different mechanisms of action. As an alkylating agent, it is believed that cyclophosphamide cross-links DNA strands, thus interfering with its replicative function (6). Whereas cyclophosphamide has not, to our knowledge, been shown to have activity against protozoan infections, related agents affected the development of malaria parasites in the mosquito host (7).

Actinomycin, on the other hand, has already been reported to show activity against some parasites, *viz., Tetrahymena pyriformis* and ciliated protozoans (8–10), trypanosomes (11, 12), *Trichomonades* (13), and the nematode, *Anquilla aceti* (14). The lack of difference between a single and double daily application of 10 μg/kg of this drug is probably due to its only being able to act on a short interval in the plasmodium's cell cycle, the S-phase, when DNA-directed RNA-synthesis can be interrupted. At the low single doses of only 10 μg/kg body weight, actinomycin D appears to be, on an equimolar basis, one of the most potent drugs ever tested against malaria, and certainly the first antibiotic to show such antimalarial activity. Considering its rapid uptake in many tissues of the body (15–17), it does not seem unlikely that this agent, as well as perhaps also cyclophosphamide, might have activity against the exoerythrocytic stages of the malaria parasites.

It should be mentioned that differences in drug sensitivity are of course present between plasmodia and tumor cells: (i) The more common antimalarials (*e.g.*, chloroquine, quinine, pyrimethamine) are not very cancerostatic, and (ii) aminopterin and amethopterin, although similar antifolics as pyrimethamine, are not similarly effective against malaria (18). Nevertheless, the good antimalarial effects shown here for cyclosphosphamide and actinomycin D stimulate further interest in these and related anticancer agents, both as possible prototypes for developing other antimalarial agents and for studying metabolic pathways of the blood and tissue stages of these parasites.

Summary. Cyclophosphamide and actinomycin D were highly active against *Plasmodium vinckei* parasitemia in NMRI mice, even more so than chloroquine. In fact, actinomycin D proved effective at the very low dose of 10 μg/kg. The efficacy of anticancer agents as antimalarials, and *vice versa,* seems worthy of further study.

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