

In Vitro Activities of the Biguanide PS-15 and Its Metabolite, WR99210, against Cycloguanil-Resistant *Plasmodium falciparum* Isolates from Thailand

M. D. EDSTEIN,^{1*} S. BAHR,¹ B. KOTECKA,¹ G. D. SHANKS,^{1,2} AND K. H. RIECKMANN¹
Australian Army Malaria Institute, Brisbane, Australia,¹ and U.S. Army Medical Research Unit—Kenya, Nairobi, Kenya²

Received 13 December 1996/Returned for modification 28 March 1997/Accepted 17 July 1997

The in vitro activities of the new biguanide PS-15 and its putative active metabolite, WR99210, were determined against seven different isolates or clones of *Plasmodium falciparum*. The mean 50% inhibitory concentrations of PS-15 and WR99210 were 1,015 and 0.06 ng/ml, respectively. WR99210 was up to 363 times more potent than cycloguanil, the active metabolite of proguanil, against cycloguanil-resistant parasites. The pronounced activity of WR99210 against multidrug-resistant *P. falciparum* indicates that further studies are required to determine the value of the prodrug, PS-15, as an antimalarial agent.

Plasmodium falciparum resistance to chloroquine and the antifolate drugs pyrimethamine-sulfadoxine (Fansidar) and proguanil is widespread in Southeast Asia (4, 9). In Thailand, resistance to mefloquine has developed rapidly over the last decade. New antimalarial drugs are urgently needed both for treatment and for prophylaxis. Recently, a biguanide related to proguanil, designated PS-15 [also known as WR250417; *N*-3-(2,4,5-trichlorophenoxypropoxy)-*N'*-(1-methyl-ethyl)imidocarbonimidic diamide], was developed as a potentially new antimalarial drug (2). PS-15 is metabolized to its putative active metabolite, WR99210 [also known as BRL6231; 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(2,4,5-trichlorophenoxypropoxy)-1,3,5-triazine], a structural analog of cycloguanil, the active metabolite of proguanil.

In 1973, Rieckmann (12) demonstrated the potent in vitro antimalarial activity of WR99210 against the highly chloroquine- and pyrimethamine-resistant strain Viet-Nam (Marks). The potency of WR99210, its lack of in vivo cross-resistance with chloroquine, pyrimethamine, and cycloguanil (6), and the relative difficulty of inducing resistance to the triazine in *Plasmodium berghei*-infected mice (7) led to clinical trials with WR99210. Oral administration of this compound was associated with gastrointestinal intolerance, possibly due to poor drug absorption (1). Nevertheless, because of the significant antimalarial activity of WR99210, it provided the impetus for the synthesis of the prodrug PS-15, which was designed to circumvent the toxic effects and limited bioavailability of WR99210 (2).

Studies with PS-15 with eight *Saimiri* monkeys indicated that the prodrug is readily metabolized to WR99210 (13). None of the monkeys developed diarrhea or other observable side effects. The similar concentrations of WR99210 in serum obtained by bioassay and high-performance liquid chromatographic analysis, coupled with the far greater susceptibility of parasites to WR99210 than to PS-15 in vitro, indicated that the metabolite was responsible for most of the antimalarial activity observed in vivo after administration of PS-15. These investigations also demonstrated that the antimalarial activity in serum was 20 to 86 times higher in monkeys receiving PS-15 than

in those receiving WR99210, indicating that the prodrug was absorbed much better from the gastrointestinal tract than WR99210.

Although WR99210 has been shown to have potent in vitro activity against different strains of *P. falciparum*, previous studies were carried out with a limited number of isolates or clones (2, 12) or in culture medium not suitable for the assessment of antifolates (3). The aim of this study was to assess the intrinsic in vitro activities of PS-15 and WR99210 in folate and *para*-aminobenzoic acid (PABA)-free medium and to compare their activities with those of proguanil and cycloguanil against a larger number of Thai isolates or clones of *P. falciparum* with diverse levels of resistance to cycloguanil, pyrimethamine, and dapsone.

Six culture-adapted isolates or clones of *P. falciparum* from Thailand were used in the study, and their activities were compared to that of the drug-sensitive D6 clone (11). The chloroquine- and pyrimethamine-resistant K1 isolate (15) and the chloroquine- and mefloquine-resistant CH12 isolate (16) had been in culture for at least 10 years. The other four isolates or clones had been adapted to culture more recently. The GA3 clone was obtained from a malaria-infected Thai patient who had failed sulfadoxine, pyrimethamine, and mefloquine (Fansimef) treatment (7a). Isolates PD-234, PD-701, and PD-728 were obtained from malaria-infected Thai soldiers who were not protected by daily proguanil-dapsone prophylaxis (14).

PS-15 hydrochloride, WR99210 hydrochloride, dapsone, and pyrimethamine were kindly supplied by Jacobus Pharmaceutical Company (Princeton, N.J.). Proguanil hydrochloride and cycloguanil were supplied by ICI Pharmaceuticals (Macclesfield, United Kingdom). The susceptibility of each isolate or clone to the antifolates was determined by the radioisotopic method of Milhous et al. (10), with minor modifications. Parasites were synchronized repeatedly with 5% sorbitol to produce experimental cultures consisting predominantly of parasites 6 to 12 h into the cycle (8). The parasite suspension (225 μ l/well) consisted of infected erythrocytes (1.5% hematocrit, 0.3 to 0.5% parasitemia) in folate and PABA-free RPMI 1640 medium (GIBCO, Grand Island, N.Y.) supplemented with 10% human serum, 25 mmol of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer per liter, 25 mmol of NaHCO₃ per liter, and gentamicin (25 mg per liter). The microculture plates were initially incubated for 48 h at

* Corresponding author. Mailing address: Australian Army Malaria Institute, Gallipoli Barracks, Enoggera QLD 4052, Australia. Phone: 61-7-33324928. Fax: 61-7-33324800.

TABLE 1. In vitro antimalarial activities of PS-15, proguanil, cycloguanil, WR99210, pyrimethamine, and dapsone against *P. falciparum* isolates or clones cultured in folate and PABA-free RPMI 1640 medium

Isolate or clone	IC ₅₀ (ng/ml) of the following antifolates ^a :						Ratio of IC ₅₀ of CYC to IC ₅₀ of WR99210
	PS-15	PROG	CYC	WR99210	PYR	DDS	
D6	70 ± 22	219 ± 39	0.06 ± 0.02	0.06 ± 0.06	0.06 ± 0.02	3.1 ± 1.2	2.3
K1	3,133 ± 1,465	3,786 ± 1,869	5.5 ± 1.4	0.12 ± 0.02	178 ± 36	11.0 ± 2.8	46
CH12	326 ± 82	608 ± 143	10.9 ± 1.5	0.05 ± 0.01	177 ± 20	13.0 ± 4.1	218
GA3	532 ± 122	844 ± 252	0.94 ± 0.38	0.02 ± 0.01	36.2 ± 12.1	195 ± 48	47
PD-234 ^b	ND	ND	17.6 ± 7.8	0.06 ± 0.04	0.47 ± 0.23	1.6 ± 0.7	293
PD-701 ^b	ND	ND	14.5 ± 2.7	0.04 ± 0.02	0.35 ± 0.08	1.3 ± 0.4	363
PD-728 ^b	ND	ND	14.8 ± 7.0	0.07 ± 0.03	0.45 ± 0.27	2.8 ± 2.0	211

^a PROG, proguanil; CYC, cycloguanil; PYR, pyrimethamine; DDS, dapsone; ND, not determined. Mean ± standard deviation IC₅₀ vs are based on the results of three or more experiments.

^b Isolates of *P. falciparum* obtained from Thai soldiers while on proguanil-dapsone prophylaxis.

37.5°C in a gas mixture of 5% O₂-5% CO₂-90% N₂ to allow for merozoite reinvasion of the erythrocytes. [³H]hypoxanthine (25 µl containing 0.5 µCi) was then added to each well. After a further 18 h of incubation, the cells were harvested to measure the level of incorporation of [³H]hypoxanthine as an index of parasite growth inhibition. The 50% inhibitory concentration (IC₅₀) was defined as the drug concentration that inhibits 50% of isotope incorporation by the parasites relative to the level of incorporation by parasites in drug-free control wells. The concentration-response data were analyzed by linear regression analysis. Each test was repeated at least three times.

The in vitro activities of PS-15, proguanil, cycloguanil, WR99210, pyrimethamine, and dapsone against isolates or clones of *P. falciparum* are given in Table 1. PS-15 showed poor antimalarial activity against the D6 and GA3 clones and the K1 and CH12 isolates and was not appreciably more active than proguanil. WR99210 was more than 1,000 times more active than PS-15 and was also 46 to 363 times more active than cycloguanil against all isolates or clones except the cycloguanil-sensitive D6 clone. Noteworthy was the marked activity of WR99210 against the three isolates obtained from the malaria-infected Thai soldiers while on proguanil-dapsone prophylaxis.

Our findings indicate that WR99210 is remarkably active against older and newer parasite isolates from Thailand, where current antimalarial agents are often no longer effective against falciparum malaria. The lack of cross-resistance with cycloguanil and pyrimethamine suggests that the mechanism of action of WR99210 differs from those of other antifolates. The data support those obtained with old clones or isolates from other countries (2, 12) where parasites generally remain more susceptible to antimalarial drugs than in Thailand.

No definitive toxicity studies have yet been carried out with PS-15. However, drug-induced toxicity was not seen in rodents (5) or *Saimiri* monkeys given PS-15 (13). If humans are able to tolerate, absorb, and metabolize PS-15, the substantial in vitro activity of WR99210 against multidrug-resistant *P. falciparum* suggests that PS-15 is a prime candidate for further development as an antimalarial drug.

We thank Kyle Webster and Dennis Kyle for providing clones D6 and GA3 and the CH12 isolate.

This paper is published with the permission of the Director General of Army Health Services.

REFERENCES

1. Canfield, C. J. 1986. New antimalarials under development, p. 99-100. In L. J. Bruce-Chwatt (ed.), *Chemotherapy of malaria*, 2nd ed. World Health

Organization Monograph Series no. 27. World Health Organization, Geneva, Switzerland.

2. Canfield, C. J., W. K. Milhous, A. L. Ager, R. N. Rossan, T. R. Sweeney, N. J. Lewis, and D. P. Jacobus. 1993. PS-15: a potent, orally active antimalarial from a new class of folic acid antagonists. *Am. J. Trop. Med. Hyg.* **49**:121-126.
3. Childs, G. E., and C. Lambros. 1986. Analogues of *N*-benzyloxydihydrotriazine: in vitro antimalarial activity against *Plasmodium falciparum*. *Ann. Trop. Med. Parasitol.* **80**:177-181.
4. Harinasuta, T., and D. Bunnag. 1988. Management of malaria with special reference to drug resistance. *Jpn. J. Trop. Med. Hyg.* **16**:121-130.
5. Hughes, W. T., D. P. Jacobus, C. Canfield, and J. Killmar. 1993. Anti-*Pneumocystis carinii* activity of PS-15, a new biguanide folate antagonist. *Antimicrob. Agents Chemother.* **37**:1417-1419.
6. Knight, D. J., and P. Mamalis. 1982. The antimalarial activity of *N*-benzyloxydihydrotriazines. III. The activity of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(2,4,5-trichloropropoxy)-1,3,5-triazine hydrobromide (BRL 51084) and hydrochloride (BRL 6231). *Ann. Trop. Med. Parasitol.* **76**:1-7.
7. Knight, D. J., and P. Williams. 1982. The antimalarial activity of *N*-benzyloxydihydrotriazines. IV. The development of resistance to BRL 6231 (4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(2,4,5-trichloropropoxy)-1,3,5-triazine hydrochloride) by *Plasmodium berghei*. *Ann. Trop. Med. Parasitol.* **76**:9-14.
- 7a. Kyle, D. E. Unpublished data.
8. Lambros, C., and J. P. Vanderberg. 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J. Parasitol.* **65**:418-420.
9. Limsomwong, N., L. W. Pang, and P. Singharaj. 1988. Malaria prophylaxis with proguanil in children living in a malaria-endemic area. *Am. J. Trop. Med. Hyg.* **38**:231-236.
10. Milhous, W. K., N. F. Weatherly, J. H. Bowdre, and R. E. Desjardins. 1985. In vitro activities of and mechanisms of resistance to antifol antimalarial drugs. *Antimicrob. Agents Chemother.* **27**:525-530.
11. Oduola, A. M. J., N. F. Weatherly, J. H. Bowdre, and R. E. Desjardins. 1988. *Plasmodium falciparum*: cloning by single-erythrocyte micromanipulation and heterogeneity in vitro. *Exp. Parasitol.* **66**:86-95.
12. Rieckmann, K. H. 1973. The in vitro activity of experimental antimalarial compounds against strains of *Plasmodium falciparum* with varying degrees of sensitivity to pyrimethamine and chloroquine, p. 58. In *Chemotherapy of malaria and resistance to antimalarials*. World Health Organization Technical Report Series no. 529. World Health Organization, Geneva, Switzerland.
13. Rieckmann, K. H., A. E. T. Yeo, and M. D. Edstein. 1996. Activity of PS-15 and its metabolite, WR99210, against *Plasmodium falciparum* in an in vivo-in vitro model. *Trans. R. Soc. Trop. Med. Hyg.* **90**:568-571.
14. Shanks, G. D., M. D. Edstein, V. Suriyamongkol, S. Timsaad, and H. K. Webster. 1992. Malaria chemoprophylaxis using proguanil/dapsone combinations on the Thai-Cambodian border. *Am. J. Trop. Med. Hyg.* **46**:643-648.
15. Thaitong, S., and G. H. Beale. 1981. Resistance of ten Thai isolates of *Plasmodium falciparum* to chloroquine and pyrimethamine by in vitro tests. *Trans. R. Soc. Trop. Med. Hyg.* **75**:271-273.
16. Webster, H. K., E. F. Boudreau, K. Pavanand, K. Yongvanitchit, and L. W. Pang. 1985. Antimalarial drug susceptibility testing of *Plasmodium falciparum* in Thailand using a microdilution radioisotopic method. *Am. J. Trop. Med. Hyg.* **34**:228-235.