Antimalarial Activity of Tetrahydrohomopteroic Acid

Abstract. *Tetrahydrohomopteroic acid, an analog of tetrahydrofolic acid, shows antimalarial activity against* Plasmodium cynomolgi *and a pyrimethamine-resistant variant of this organism.*

Tetrahydrohomofolic acid (Fig. 1) is an analog of tetrahydrofolic acid (*1*), which has the ability to inhibit the growth of bacteria and tumors that are resistant to amethopterin (*2*). While the potency of this compound as an antimalarial agent was being assayed, it was observed that crude preparations possessed activity qualitatively comparable to pyrimethamine, whereas purified material was inactive. A significant impurity in the active preparation was tetrahydrohomopteroic acid (Fig. 1). We now report tests on the antimalarial activity of this compound.

Homopteroic acid was supplied by the Cancer Chemotherapy National Service Center. It was neutralized with NaOH to *p*H 8.8 and hydrogenated to tetrahydrohomopteroate in the presence of platinum catalyst. The product was lyophilized and stored in a vacuum. When dissolved in 0.2*M* 2-mercaptoethanol, this material exhibited a single absorption maximum at 285 mμ with insignificant absorption at 340 mμ. It caused 50 percent inhibition of *Streptococcus faecalis* (American Type Culture Collection No. 8043) at $0.75 \times 10^{-9}M$ when assayed (*2*).

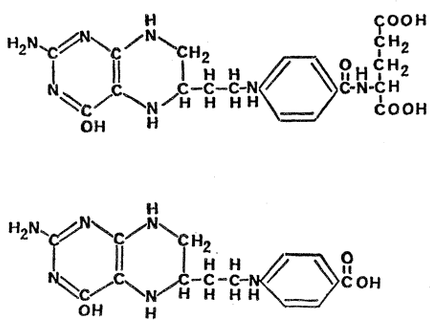

Fig. 1. Tetrahydrohomofolic acid (top) and tetrahydrohomopteroic acid (bottom).

Just prior to intravenous injection tetrahydrohomopteroic acid was dissolved in sterile water under hydrogen. To each 20 ml of a solution containing 50 mg/ml was added 0.25 ml of an ascorbic acid solution (100 mg/ml). The *p*H of the resulting solution was 7.2.

Healthy rhesus monkeys (*Macaca mulatta*) were inoculated with 500,000 trophozoites of either the RO strain (pyrimethamine-sensitive) or the RO/PM strain pyrimethamine-resistant) *Plasmodium cynomolgi* which was derived from the RO strain by the method described by Schmidt and Genther (*3*). The RO/PM strain is at least 1000 times more resistant to pyrimethamine than the RO strain is. Treatment was initiated 7 days after inoculation when 10 to 50 parasites per 10^4 erythrocytes were observed. Doses of 80 mg of tetrahydrohomopteroic acid per kilogram given daily for 5 days eradicated parasites from the blood of monkeys infected with pyrimethamine-sensitive or pyrimethamine-resistant strains of *Plasmodium cynomolgi* (Table 1). No recrudescence of parasitemia was observed. Although high values of blood urea nitrogen were observed and significant weight loss occurred, the monkeys survived and remained apparently normal. Doses of 60 and 40 mg/kg per day reduced the number of parasites so that they were not observed in the blood samples taken. Recrudescence occurred subsequently. A single dose of 80 mg/kg did not alter the course of parasitemia.

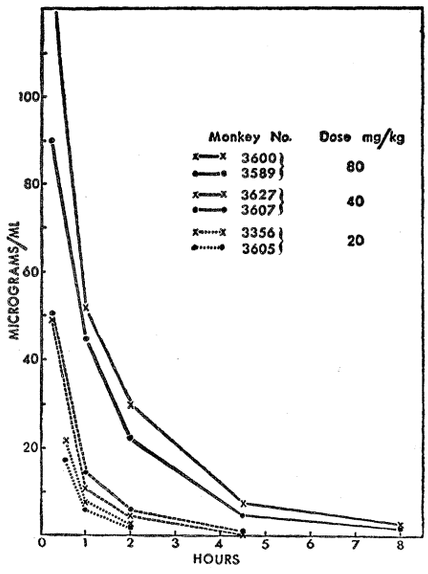

Fig. 2. Concentration of tetrahydrohomopteroic acid in the blood after intravenous injection.

Concurrent administration of tetrahydrofolate prevented the antimalarial effect of tetrahydrohomopteroic acid but did not prevent the rise in blood urea nitrogen (Table 1).

Single intravenous doses of tetrahydrohomopteroic acid to uninfected monkeys resulted in blood levels shown in Fig. 2, as determined by assay with *Streptococcus faecalis* (*2*). The initial half-time is 50 minutes, a value similar to that obtained for tetrahydrohomofolic acid in mice (*2*). At 24 hours, the drug was no longer detectable in the blood.

Table 1. Response of *Plasmodium cynomolgi* in infected monkeys to treatment with tetrahydrohomopteroic acid (THHP) and tetrahydrofolic acid (THF). The blood urea nitrogen (BUN) was measured on days 17, 25, and 33 in experiments 1 and 2, and on days 14, 19, and 25 in experiment 3.

Dose (mg kg^{-1} day^{-1})		Number of parasites per 10^4 red blood cells after inoculation on day:										BUN (mg/100 ml) on day:			Weight loss on day 17 (%)
THHP	THF	7*	9*	11*	13	15	17	19	26	40	80	17	25	33	
						Experiment 1: Pyrimethamine-sensitive organisms									
80	None	23	38	10	1	0	0	0	0	0	0	226	47	28	23
40	None	14	63	36	16	2	0	0	196			41	23	17	6
None	None	8	116	642	202	17	24	47	66	<1†	18	27			7
						Experiment 2: Pyrimethamine-resistant organisms									
80	None	27	63	5	<1	0	0	0	0	0	0	196	28	21	17
40	None	14	89	9	6	<1	0	<1	24			46	28	19	10
None	None	12	119	327	56	14	16	136	14	2	<1	25	23	22	0
												Day			
												14	19	25	
						Experiment 3: Pyrimethamine-sensitive organisms									
60	None	12	28	6	3	<1	0	0	<1			126	79	26	10
60	30	10	70	408	872	234	95	270				110	32	23	10
None	60	20	179	1500	565	55	82					25	21	20	5
None	None	10	77	920	226		41	145				22	24	14	5

* Intravenous doses for 5 consecutive days (7 to 11). † <1 Means parasites present, but fewer than one per 10^4 red blood cells.

Table 2. Effect of a single dose of tetrahydrohomopteroate on normal monkeys, measured as blood urea nitrogen (BUN) on the 8th and 12th days after injection.

Monkey	Dose (mg/kg)	BUN (mg/100 ml)	
		Day 8	Day 12
3600	80	66	35
3589	80	125	25
3627	40	146	52
3607	40	27	22
3356	20	38	22
3605	20	33	28

Eight days after the injection, monkeys 3600, 3589, and 3627 showed an elevated blood urea nitrogen (Table 2), which decreased to normal 4 days later. Monkey 3607 showed no ill effects, and in monkeys 3605 and 3356 only slight rise was detected. This effect of the drug appears to be completely reversible. After a single dose of the drug (40 mg/kg) was administered by stomach tube, detectable blood levels were not observed.

The evidence presented shows that tetrahydrohomopteroic acid is a new type of antifolate which is effective against parasites which are resistant to pyrimethamine, which is a 2, 4-diaminopyrimidine. Pyrimethamine is considered to exert its antimalarial effect by inhibiting dihydrofolate reductase (*4*). The new agent probably does not exert its major action here since it has been shown that dihydrohomopteroate serves as a substrate for mouse tumor dihydrofolate reductase (*5*). In the presence of reduced nicotinamide-adenine dinucleotide phosphate the reductase catalyzes the formation of tetrahydro- from dihydrohomopteroic acid at one half the rate observed for the reduction of dihydrofolate to tetrahydrofolate.

Sulfonamides are believed to have antimalarial activity because they inhibit the incorporation of *p*-aminobenzoic acid into dihydrofolic acid (*4*). Since tetrahydrohomopteroic acid has a *p*-aminobenzoic acid moiety it might act at the same site as the sulfonamides. The presence of the pteridine moiety in the new drug introduces the additional possibility that enzymes of dihydrofolate biosynthesis which involve pteridine but not *p*-aminobenzoic acid may be inhibited. Two examples of enzymes in this category are (i) the enzyme that catalyzes the pyrophosphorylation of 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine (*6*) and (ii) the enzyme that catalyzes the addition of glutamate to dihydropteroate (*7*).

Two other possibilities should also be considered. (i) Tetrahydrohomopteroic acid might inhibit an enzyme involved in the feedback control of folate biosynthesis; (ii) it might compete with tetrahydrofolate for coenzyme sites involved in the synthesis of essential metabolites (*8*). For example, it inhibits *Escherichia coli* thymidylate synthetase by 35 percent at $3.4 \times 10^{-5} M$ (*5*). The possibility that the drug inhibits folate metabolism at sites other than those attacked by pyrimethamine or sulfonamides suggests that combinations of these drugs should be tested for synergistic effects.

R. L. KISLIUK
M. FRIEDKIN
Department of Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111
L. H. SCHMIDT
R. N. ROSSAN
National Center for Primate Biology, University of California, Davis 95616

References and Notes

1. R. L. Kisliuk and M. Friedkin, *Int. Congr. Biochem.*, 6th, New York, 1964. *Abstr.* **1**, 65 (1964); L. Goodman, J. DeGraw, R. L. Kisliuk, M. Friedkin, E. J. Pastore, E. J. Crawford, L. T. Plante, A. Al-Nahas, J. F. Morningstar, Jr., G. Kwok, L. Wilson, E. F. Donovan, J. Ratzan, *J. Amer. Chem. Soc.* **86**, 308 (1964); J. DeGraw, J. P. Marsh, Jr., E. M. Acton, O. P. Crews, C. Mosher, A. N. Fujiwara, L. Goodman, *J. Org. Chem.* **30**, 3404 (1965).
2. J. A. R. Mead, A. Goldin, R. L. Kisliuk, M. Friedkin, L. Plante, E. J. Crawford, G. Kwok, *Cancer Res.* **26**, 2374 (1966).
3. L. H. Schmidt and C. S. Genther, *J. Pharmacol. Exp. Therap.* **107**, 61 (1953).
4. R. Ferone and G. H. Hitchings, *J. Protozool.* **13**, 504 (1966).
5. L. T. Plante, E. J. Crawford, M. Friedkin, *J. Biol. Chem.* **242**, 1466 (1967).
6. T. Shiota, M. N. Disraely, M. P. McCann, *ibid.* **239**, 2259 (1964).
7. G. M. Brown, R. A. Weisman, D. A. Molnar, *ibid.* **236**, 2534 (1964).
8. R. L. Kisliuk and M. D. Levine, *ibid.* **239**, 1900 (1964).
9. Supported by NIH grants FR 00169, AIO 5888, CA-05997, GM-1187102; NSF grant GB-1890; and a professional service contract from the Cancer Chemotherapy National Service Center. We thank Miss E. J. Crawford, Mrs. J. Harrison, and Miss D. Lewis for assistance.

22 March 1967

Pesticide Transformation to Aniline and Azo Compounds in Soil

Abstract. *The herbicide 3′,4′-dichloropropionanilide decomposes in soil to carbon dioxide and 3,4-dichloroaniline, and two molecules of the latter compound are condensed to form 3,3′,4,4′-tetrachloroazobenzene. Soil microorganisms are involved in both transformations.*

The herbicide 3′,4′-dichloropropionanilide (DCPA) was transformed by soil microorganisms; it was suggested that the aliphatic side chain of the molecule was oxidized in part to carbon dioxide and that the aromatic moiety was liberated as a toxic residue that depressed soil respiration (*1*). To establish this possibility as fact and to enable the isolation and identification of metabolites, we treated 500 g of soil (Nixon sandy loam, *p*H 5.3) with 1.0 g of DCPA, moistened it to 60 percent of capacity, and incubated it at 28°C for 17 days. By the extraction and fractionation procedures summarized below, two decomposition products of the herbicide were concentrated, crystallized, and characterized chemically as 3,4-dichloroaniline (DCA) and 3,3′,4,4′-tetrachloroazobenzene (TCAB).

For isolation of DCA, the soil was extracted with 2 liters of acetone. The filtered extract was diluted with three volumes of water, adjusted to *p*H 11.0 with NaOH, saturated with NaCl, and partitioned with hexane. The hexane fraction was extracted with 1.0*N* HCl saturated with NaCl, and the acid extract was adjusted to *p*H 11.0 with NaOH and then partitioned with chloroform. The solvent was evaporated to dryness, and the residue was recrystallized twice from ligroin and established as identical with an authentic sample of DCA by comparison of their movements on thin-layer plates (Eastman Chromagram type K301R developed with benzene; R_F values: DCPA, 0.20, DCA, 0.67, and TCAB, 0.94), by retention times in a gas chromatograph (Aerograph 660, 1.5 meters long and 0.3 cm outside diameter stainless steel column, packed with 5 percent SE-30 on Chromosorb W; carrier: 60 ml N_2 per minute; flame ionization detector; retention at 155°C, DCPA, 5 minutes, and DCA, 1 minute; retention at 200°C, DCPA, 1 minute, and TCAB, 6 minutes), by infrared spectra, and by melting points (71°C).

For the isolation of TCAB, the hexane-soluble material extracted from soil was evaporated to dryness, and the residue was dissolved in benzene and applied to a silica-gel column. Elution with benzene yielded a fast-moving orange fraction which was purified by