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Antimalarial Activities of Triazine Metabolites of Chlorguanide and Dichlorguanide.* (19625)

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During a study of the metabolic fate of the antimalarial drug chlorguanide, Carrington and coworkers(1) isolated a compound from the urine of humans and the feces of rabbits which was believed to be the hypothetical "active metabolite"(2-4) of the above drug. The isolated compound, tentatively identified as 2,4-diamino-1-p-chlorophenyl-1,6-dihydro-6,6-dimethyl-1,3,5-triazine (*CPT*), was reportedly 10 times as active as chlorguanide against infections with *Plasmodium gallinaceum* in the chick. These workers also described a second compound, 2,4-diamino-1-(3, 4-dichlorophenyl)-1, 6-dihydro-6, 6-dimethyl-1.3,5-triazine (*DCPT*), which was considered (5) to be the corresponding metabolite of dichlorguanide(6) and which was 50 times as active as the latter drug and 100 times as active as chlorguanide against infections with the above plasmodium(1,5).†

These findings were of considerable interest to this laboratory, which since 1946 has studied the metabolic fate of chlorguanide and related biguanides in man and rhesus monkey. Although this investigation(8,9) had pointed to the pattern of metabolic degradation described by Carrington, it had failed to provide any evidence for the presence in urine, feces, sera or tissues of metabolites more active than their parent drugs. Since this conclusion was based on assessments of activity in *P. cynomolgi* infections in the rhesus monkey(9), whereas that of Carrington and Davey was based on work with *P. gallinaceum*, the question arose whether the divergent findings were related to the use of different test objects. This led to a study of the activities of the triazines, *CPT* and *DCPT*, against infections with *P. cynomolgi*, the results of which are presented in this report.

Materials and methods. The comparative antimalarial activities of *CPT* and chlorguanide were measured in a group of 18 rhesus monkeys (3.5 to 4.8 kg), each of which was inoculated intravenously with approximately 500,000 trophozoites, obtained by suitable dilution in 0.85% saline of the blood of a passage monkey infected with the stock strain of *P. cynomolgi*.‡ Through use of conventional thick and thin blood films stained with Giemsa, parasitemias were followed daily until a density of approximately 100 parasites per 10,000 erythrocytes was attained. At this point, groups of 8 monkeys were treated either with *CPT* or with chlorguanide, the 2 remaining animals serving as untreated controls. Because previous studies(9) had shown that absorption of orally administered dihydrotriazines was uncertain, each drug was adminis-

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† The activities of these triazines against *P. gallinaceum* infections have been confirmed recently by Coatney(7).

‡ The strain of *P. cynomolgi* was obtained June 1946 from Dr. R. J. Porter, University of Michigan. Since that time it has been maintained by serial intravenous trophozoite passages through untreated rhesus monkeys at intervals of 2 to 4 weeks.

tered to 4 monkeys by stomach tube and to 4 intramuscularly. Solutions of these drugs in 0.85% saline were employed for all treatments. These solutions were prepared immediately prior to administration in order to eliminate the problem of triazine rearrangement (1,5,9). The doses of chlorguanide used were arranged on the basis of previous large-scale experience(10,11) to provide subeffective, minimally-effective, parasitemia-suppressive, and curative dosage levels. Identical doses of *CPT* were employed in order to detect either enhanced or reduced activity. In all instances the drugs were administered once daily (8:30 A.M.) for 7 consecutive days. Parasitological studies were made daily throughout treatment and for at least 10 days thereafter, unless parasitemias were abolished. In the latter instance, blood examinations were made once weekly either until a recrudescence occurred or until evidence was obtained that the infection had been cured.§ The study of the comparative activities of *DCPT* and chlorguanide followed the above pattern in all important respects. The only differences were the use of 20 monkeys instead of 18, to provide 4 untreated controls, and the administration of smaller doses of *DCPT* than chlorguanide, in order to detect the enhanced activity of the dichlorguanide metabolite anticipated from the results of the avian studies of Carrington and Davey(1,5).‖

Results. The data on the relative activities of *CPT* and chlorguanide, summarized in Table I, indicate clearly that the parent biguanide is more effective than its metabolite against infections with *P. cynomolgi.* The small number of monkeys used in this study makes it impossible to appraise this activity difference precisely. However, a rough comparison, based on the doses of the respective drugs required to effect minimal but significant reductions in parasitemia, suggests that chlorguanide is at least twice and possibly 4 times as active as *CPT.* Thus when administered intramuscularly, 0.075 mg per kg doses of *CPT* were required for the same effects as were produced by 0.0188 mg per kg doses of chlorguanide (cf. M-4872 and M-4892). Administered orally, 0.3 mg doses of *CPT* and 0.075 mg doses of chlorguanide gave comparable results (cf. M-4874 and M-4888).

The data in Table I also show that *DCPT* was somewhat more active than *CPT.* However, the activity of *DCPT,* the dichlorguanide metabolite, was no greater than that of chlorguanide. This is shown clearly by a comparison of the doses of these compounds required for minimal but significant effects on parasitemia. These doses were identical for the two compounds, 0.0188 mg per kg, intramuscularly (cf. M-4745 and M-4730) and 0.075 mg per kg, orally (cf. M-4775 and M-4726).

Discussion. From the data presented above

§ Infections were considered cured when there was no recurrence of parasitemia within 12 weeks after treatment despite attempted provocation of relapse by splenectomy.

‖ Since the triazine metabolites, *CPT* and *DCPT,* are not generally available and since their structures have not been established conclusively, it is important to record the sources and characteristics of the compounds used in this study. *DCPT* was obtained through the generosity of Dr. D. G. Davey, Biological Laboratories, Imperial Chemical (Pharmaceuticals) Ltd., Wilmslow, England. This compound was supplied as the monohydrochloride, m.p. 190° uncorrected. *Analysis.* Calculated for $C_{11}H_{13}Cl_2N_5 \cdot HCl$: C, 40.95; H. 4.37; Cl, 32.97; N, 21.71. Found: C, 41.39; H, 4.21; Cl, 32.77; N, 21.22. According to ultraviolet absorption measurements(1,9) this substance was converted quantitatively to 4-amino-2-(3,4-dichloroanilino)-1,6-dihydro-6, 6-dimethyl-1,3,5-triazine by heating in 2 N sodium hydroxide at 100° for 20 minutes. The hydrated dihydrochloride of *CPT* was synthesized in our laboratories(12) by refluxing a concentrated hydrochloric acid solution of p-chlorophenylbiguanide with acetone, m.p. 216-21°, uncorrected. *Analysis.* Calculated for $C_{11}H_{14}ClN_5 \cdot 2\,HCl \cdot 2\,H_2O$: N, 19.42. Found: N, 19.94. The monohydrochloride (m.p. 200-207°) and picrate (m.p. 215-218°) of *CPT* were also prepared. *Analysis.* Calculated for $C_{11}H_{14}ClN_5 \cdot HCl$: C, 45.84; H, 5.25. Found: C, 45.64; H, 5.09. Calculated for $C_{11}H_{14}ClN_5 \cdot C_6H_3N_3O_7$: C, 42.46; H, 3.56; N, 23.31. Found: C, 42.72; H, 3.13; N, 22.68. Ultraviolet absorption studies showed that *CPT,* like *DCPT,* was converted quantitatively to the corresponding anilino isomer (4-amino-2-p-chloroanilino-1, 6-dihydro-6,6-dimethyl-1,3,5-triazine) by heating in 2 N sodium hydroxide at 100° for 20 minutes(1,9). The chlorguanide monohydrochloride used in current experiments was provided by Dr. Davey in 1946. According to countercurrent distribution pattern, the homogeneity of this preparation was in excess of 98%.

TABLE I. Comparative Antimalarial Activities of Chlorguanide, 2,4-diamino-1-p-chlorophenyl-1,6-dihydro-6,6-dimethyl-1,3,5-triazine (*CPT*), and 2,4-diamino-1-(3,4-dichlorophenyl)-1, 6-dihydro-6, 6-dimethyl-1,3,5-triazine (*DCPT*).

Drug and route of adminis.	Monkey No.	Daily dose, mg base/kg body wt	No. parasites/10000 erythrocytes — Day of treatment 0	1	2	3	4	5	6	Day after treatment 1	2	3	4	Subsequent course of infection†
CPT, intramusc.	4865	.0047	120	84	370	540	1010	600	130	62	34	16	4	P
	78	.0188	200	360	1700	2100	1240	200	96	55	67	120	160	P
	72	.075	75	59	51	47*	56*	75*	21*	1	<1	<1	<1	R
	67	.3	140	150*	82*	1*	2	2	1	1	—	—	—	R
Chlorguanide, intramusc.	87	.0047	220	390	1390	1430	930	310	170	48	88	63	73	P
	92	.0188	150	230	420	400*	300*	180*	49*	8*	3	2	1	P
	96	.075	190	160*	120*	4*	6*	1	2	<1	—	—	<1	R
	98	.3	180	140*	54*	4*	7*	5*	3*	<1	—	—	—	C
CPT, oral	80	.0188	110	220	1120	1010	770	210	460	280	200	140	110	P
	71	.075	170	360	660	860	730	320	110	37	27	23	91	P
	74	.3	86	92*	18*	3*	1	1	1	<1	—	<1	<1	R
	84	1.25	120	110	47*	4*	10*	1	1	<1	—	—	—	C
Chlorguanide, oral	69	.0188	130	190	350	420	250	160	170	30	30	10	10	P
	88	.075	130	170	210*	120*	32*	14*	4*	1	2	<1	<1	R
	89	.3	110	100*	69*	5*	1*	2	1	<1	<1	—	—	R
	77	1.25	170	180*	120*	9*	6*	3*	2	2	—	—	—	C
Controls	63	0	110	170	200	610	550	440	190	43	18	15	7	
	76	0	79	190	600	1330	780	420	360	59	46	45	86	
DCPT, intramusc.	4770	.0012	82	260	420	900	1130	830	530	320	130	120	70	P
	32	.0047	110	230	760	1010	650	240	67	40	77	96	140	P
	45	.0188	81	120*	45*	5*	7*	1	1	5	15	19	99	P
	73	.075	130	80*	17*	6*	1*	1*	1	<1	—	—	—	R
Chlorguanide, intramusc.	19	.0047	56	140	740	440	520	98	66	16	59	59	31	P
	30	.0188	14	110	420*	120*	150*	7*	5*	<1	<1	<1	<1	R
	17	.075	8	18	22*	6*	4*	3*	<1	<1	<1	<1	<1	R
	34	.3	11	59	87*	7*	4*	2*	2*	<1	<1	<1	—	C
DCPT, oral	60	.0047	120	520	1130	2100	1650	750	270	59	45	130	100	P
	80	.0188	160	420	1340	1390	1570	830	140	85	69	150	320	P
	75	.075	130	330	460	260*	100*	36*	3*	3*	1*	<1	<1	R
	71	.3	110	98*	52*	5*	2*	3*	2*	<1	<1	2	7	R
Chlorguanide, oral	41	.0188	17	100	580	1050	950	490	140	62	65	150	190	P
	26	.075	13	25	19*	9*	4*	2*	2*	<1	<1	<1	<1	R
	20	.30	82	120	57*	12*	10*	8*	3*	<1	<1	<1	<1	R
	38	1.25	66	120	170*	8*	3*	1*	<1	<1	—	—	—	C
Controls	43	0	55	82	940	1200	1080	380	170	7	32	20	47	
	52	0	160	600	820	1610	1470	630	85	86	92	120	160	
	68	0	86	320	770	1340	1140	550	170	84	100	91	170	
	82	0	20	25	300	330	650	630	950	370	230	15	10	

* Abnormal schizonts, with chromatin scattered diffusely throughout parasite, predominate.

† P = infection persisted, following pattern of untreated infection; R=spontaneous recrudescence of infection; C = cure, or eradication, evidenced by absence of parasitemia for 12 wk despite splenectomy.

it is evident that the activities of the triazine metabolites of chlorguanide and dichlorguanide against *P. cynomolgi* infections in the rhesus monkey are in no way comparable to the activities of these compounds against *P. gallinaceum* infections in the chick. Whereas in this avian disease *CPT* and *DCPT* were respectively 10 and 100 times as effective as chlorguanide, in the simian infection *CPT* had only one-half to one-fourth the activity of the parent biguanide, *DCPT* equal activity. At present there is no satisfactory explanation for the divergent responses of the above infections. Such diverse reactions are not uncommon, however, in malaria chemotherapy(13). In this instance, they may rest either on inherent differences in the susceptibilities of *P. cynomolgi* and *P. gallinaceum* to the specific compounds, or to differences in the physiological disposition of the metabolites in the

chick and monkey, the former possibility being the more likely. In any case, the demonstration that *CPT* is distinctly less active than chlorguanide against infections with *P. cynomolgi* explains previous failures to isolate the hypothetical "active metabolite" of chlorguanide.

In conclusion some comment on the practical implications of the current findings should be made. The high activities of CPT and especially *DCPT* against infections with *P. gallinaceum* might suggest that these drugs would be a striking improvement over chlorguanide in the treatment of human malaria. Such optimism is not justified by the findings in *P. cynomolgi* infections, which in a wide experience have proved to be a highly reliable indicator of the value of drugs against human malaria. The demonstrations that in the simian disease *CPT* is distinctly less effective than chlorguanide and that *DCPT* has essentially the same effectiveness as this biguanide suggest that with respect to inherent antimalarial activity these metabolites would have little to offer in the treatment of human malaria.

Summary. The activities of the triazine metabolites of chlorguanide and dichlorguanide have been studied in *P. cynomolgi* infections in the rhesus monkey. The results show that the chlorguanide metabolite, *CPT,* has only one-half to one-fourth the activity of the parent drug, whereas the dichlorguanide metabolite, *DCPT,* has activity equal to that of chlorguanide. These findings are in marked contrast to reported results on *P. gallinaceum* infections in the chick, against which *CPT* and *DCPT* are respectively 10 and 100 times as effective as chlorguanide.

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Occurrence of Acute Pulmonary Edema in Experimental Alkalosis. (19626)

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In previous studies of experimentally induced tetany(1,2), it was found that venoclysis of solutions of sodium carbonate and sodium bicarbonate for the production of alkalotic tetany in cats frequently resulted in the appearance of gross lung edema. This edema resembled that produced by the administration of toxic doses of ammonium salts previously described and studied by several of us(3,4). The purpose of this report is to describe the experimental production of acute pulmonary edema with the alkaline salts of sodium together with some preliminary observations on the mechanism of its production.

Material and methods. The data presented in this paper are based on the study of 25 adult cats anesthetized with sodium pentobarbital (35 mg/kg). The trachea was cannulated in all experiments. In a number of experiments artificial respiration was provided

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