Activities of Trimethoprim against Infections with Pyrimethamine Susceptible and Resistant Strains of *Plasmodium cynomolgi** (33861)

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Recent studies in human volunteers by Martin and Arnold (1, 2) have directed attention to trimethoprim [2,4-diamino-5-(3′,4′,5′-trimethoxybenzyl)-pyrimidine] as a potentially useful antimalarial drug. These investigations indicated that this folic acid antagonist was able to eradicate trophozoite-induced infections with the drug susceptible Uganda I strain of *Plasmodium falciparum* and at slightly higher doses effected rapid control of infections induced by the pyrimethamine–chloroquine resistant Malayan Camp strain. The responses of infections with the latter parasite were unexpected since with both trimethoprim and pyrimethamine functioning as dihydrofolic acid reductase inhibitors (3, 4), cross resistance would have been anticipated.

In view of the likely application of trimethoprim, either alone or in combination with other agents, to treatment of human malaria infections, further study of cross resistance seemed worthwhile. As reported here, this has been done by quantitative evaluation of the capacities of trimethoprim to control established infections with pyrimethamine susceptible and resistant strains of the simian parasite, *Plasmodium cynomolgi*. These strains have previously been used successfully (5, 6) to identify similarities and differences in antimalarial properties of various folic acid antagonists.

Methods. The RO and RO/PM strains of *P. cynomolgi* were used in the current study. The RO strain, isolated in 1960 (7), is fully susceptible to all commonly used antimalarial drugs. Since isolation, it has been maintained by serial monkey to mosquito to monkey transfers[1] at approximate 8-week intervals. The RO/PM strain is fully resistant to the maximum tolerated doses of pyrimethamine, proguanil, and cycloguanil, but is wholly susceptible to such drugs as quinine, quinacrine, chloroquine (and other 4-aminoquinolines), sulfadiazine, and other sulfonamides. This strain was derived from a rhesus monkey challenged with the RO strain and tested *ad seriatim* with increasing doses of pyrimethamine until the limit of tolerability to this pyrimidine was approached. From 1961 to the present, the RO/PM strain has been maintained by serial trophozoite passages through untreated rhesus monkeys at 4-week intervals. The pyrimethamine resistance of this parasite has been examined repeatedly during this 8-year period and has remained unchanged.

Twenty-eight well conditioned, parasite negative, rhesus monkeys (*Macaca mulatta*) of Indian origin, each weighing from 4.0 to 5.5 kg, were employed. Fourteen of these animals were inoculated intravenously with approximately 5×10^5 trophozoites of the RO strain; 14 were challenged with a similar number of trophozoites of the RO/PM strain. On the day following challenge, and daily thereafter, thick and thin blood films were prepared and stained with Giemsa. When these films became positive, parasite populations were quantitated both with respect to numbers of parasites per 10^4 erythrocytes and to numbers of the various developmental stages per 100 parasites.

Twelve animals of each group of 14 were assigned to various chemotherapy regimens; 2 served as untreated controls. Therapy was initiated when the parasitemias approximated 50–100 per 10^4 erythrocytes. Of the 12 animals, 3 were given pyrimethamine via stomach tube in daily doses of 0.6 mg/kg for 7

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[1] These transfers were effected through Indian rhesus monkeys and *Anopheles freeborni*.

consecutive days. Subgroups of 3 each of the remaining 9 subjects were given trimethoprim[2] via the same route in doses of 25, 50, or 100 mg/kg, once daily for 7 days.[3] Daily parasitologic examinations were continued for at least 14 days after the last dose of pyrimethamine or trimethoprim.

Pyrimethamine was administered as the water soluble hydrochloride. Trimethoprim, relatively water insoluble, was suspended in aqueous medium with the assistance of Tween 80.

Results. The data in the upper section of Table I show that trimethoprim, at daily doses of 100 mg/kg, was able to eradicate infections resulting from challenge with the susceptible RO strain. Doses of 50 mg/kg effected temporary clearance of parasites from thick films in one of 3 subjects and permanent clearance in the other two. Doses of 25 mg/kg effected temporary parasite clearance in one individual and reduced the parasitemia in the remaining two. Thus it is clear that trimethoprim had significant activity against a pyrimethamine susceptible parasite, the responsiveness of the RO strain to pyrimethamine being reaffirmed by the data on Monkeys 5932, 5946, and 5950. The rate of action of trimethoprim at doses of 50 and 100 mg/kg was about equal to that of pyrimethamine at a dose of 0.6 mg/kg. Arrest of schizogony was usually evident on thin films within 48 hr of the first drug dose. Clearance of the "arrested" forms took place slowly and steadily thereafter.

The data in the lower half of Table I show that trimethoprim had comparatively little activity against infections with the pyrimethamine resistant RO/PM strain (cf. data on Monkeys 5912, 5919, and 5944 for evidence of pyrimethamine resistance). Parasitemias were not eliminated even temporarily by daily doses of trimethoprim up to and including 100 mg/kg. However, the morphologic data on Monkeys 5579 and 5864 indicate that this compound had a marginal effect on parasite multiplication at the topmost dose. This effect was barely equal to and probably less than that exhibited when infections with the sensitive strain were exposed to daily doses of 25 mg/kg.

Discussion. The data presented above show clearly that trimethoprim at maximum tolerated doses has little effect on infections induced by a strain of *P. cynomolgi* that is fully resistant to pyrimethamine. Thus insofar as infections with this simian plasmodium are concerned there is clearly cross resistance between these two folic acid antagonists. Although this conclusion might seem at odds with the findings of Martin and Arnold (1, 2) this is really not so. Whereas these authors stated that "Trimethoprim will cure a multiresistant *P. falciparum* infection at doses which are tolerated by the patient," the data reported show that this compound was considerably less effective against infections with the pyrimethamine–chloroquine resistant Malayan Camp strain than against infections with the all-drug susceptible Uganda I strain. The total doses of trimethoprim required for near equivalent effects against infections with the Malayan Camp and Uganda I strains were 10.5 and 3.75 g, respectively. The larger of these amounts approximates the maximum tolerated dose for man (1).

Recognition that there is a substantial level of cross resistance between trimethoprim and pyrimethamine raises questions concerning the usefulness of the former agent in areas where there are multidrug resistant strains of *P. falciparum*. It is likely that this usefulness will be determined primarily by the level of pyrimethamine resistance that prevails in the regions where application of trimethoprim is attempted. If the strains are no more resistant to this latter pyrimidine than the Malayan Camp, trimethoprim therapy may be effective. If the strains have a level of resistance comparable to that of the RO/PM line of *P. cynomolgi*, administration of trimethoprim would be of little value. A second factor that may determine the future

[2] The trimethoprim used in these studies was provided by Dr. D. Jacobus of the Walter Reed Army Institute of Research.

[3] Preliminary studies indicated that 100 mg/kg was the maximum tolerated dose of trimethoprim for the rhesus monkey in a 7-day regimen.

utility of trimethoprim is the potential for enhancing its antimalarial activity by concomitant administration with a sulfonamide. Results of a preliminary study by Martin and Arnold (2) indicate that this is a reasonable possibility.

Summary. A systematic comparison of the capacity of trimethoprim to control established infections with the drug susceptible RO and primethamine resistant RO/PM strains of *P. cynomolgi* showed that the above pyrimidine has a significant order of

TABLE I. The Activities of Trimethoprim against Infections with the Pyrimethamine Susceptible (RO) and Pyrimethamine Resistant (RO/PM) Strains of *P. cynomolgi.*

Therapy			No. of parasites/10^4 erythrocytes							Remarks[b]	
			During treatment (day)				Post-treatment (day)				
Drug[a]	Daily dose (mg/kg of body wt)	Monkey no.	0	2	4	7	2	4	6	Drug forms (day of R_x)	Recrudescence (day post-R_x)
			Subjects infected with the RO strain								
None	0	5969	26	342	1040	448	33	45	184		
		5971	58	555	1160	110	19	16	26		
TMP	25	5924	48	226	254	19	13	200	336	3	
		5928	57	80	69	2	<1	—	—	2	12
		5936	41	136	218	19	18	268	158	6	
	50	5770	82	182	29	<1	—	—	—	2	None
		5827	45	160	32	<1	—	—	—	2	None
		5844	29	93	34	<1	—	—	—	2	8
	100	5769	37	79	30	<1	—	—	—	2	None
		5818	58	520	41	<1	—	—	—	3	None
		5863	66	126	27	<1	—	—	—	2	None
PM	0.6	5932	59	80	25	1	—	—	—	2	None
		5946	44	105	24	<1	—	—	—	2	None
		5950	62	93	33	<1	—	—	—	2	14
			Subjects infected with the RO/PM strain								
None	0	5918	82	357	416	178	93	115	152		
		5926	84	404	202	11	24	43	43		
TMP	25	5549	51	224	612	35	214	327	6		
		5600	103	318	146	14	46	105	64		
		5948	61	412	515	150	480	190	14		
	50	5802	77	218	16	107	167	48	6		
		5829	131	805	262	3	7	64	118		
		5845	155	525	530	25	79	327	7		
	100	5579	103	194	85	1	1	54	92	3	
		5858	132	1150	1210	16	113	256	6		
		5864	94	111	57	1	2	22	13	3	
PM	0.6	5912	66	532	944	127	91	244	456		
		5919	106	1010	1160	963	174			Assigned to special R_x	
		5944	92	420	204	21	28	146	55		

[a] TMP = trimethoprim; PM = pyrimethamine.

[b] The term "drug forms" is applied to those schizonts with acidophilic staining cytoplasm and diffusely distributed chromatin. These forms are found as a prelude to control of parasitemia by such antimalarials as the sulfonamides, proguanil, cycloguanil, and pyrimethamine, all folic acid antagonists. Observation period, 14 days post-treatment.

activity against infections with the RO strain but, at the maximum tolerated dose, little capacity to control infections with the RO/PM. Thus contrary to expectations, based on results of earlier studies in bacterial systems and in human infections with *P. falciparum,* there is a considerable degree of cross resistance between trimethoprim and pyrimethamine.

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Absence of Infectious Virus from a Line of SV40-Transformed Human Liver Cells (33862)

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Morphologic transformation of human cells by simian virus 40 (SV40) has been the subject of a number of reports. Several of these were mentioned in a previous communication (1). More recent studies include the SV40-induced transformation of human cells derived from foreskin (2), astrocytes (3), amnion (4, 5) and the parathyroid glands (6). Certain properties have been found in these studies which distinguish transformed human cells from cells of other species transformed by SV40. These include: (a) a "crisis" period occurring at a variable time after the onset of transformation and evidenced by a decline in cell proliferation and the eventual ability to persist in serial subculture (7, 8). (b) the yielding of virus, detectable in the supernatant, before crisis. (c) a small fraction (0.01–5%) of cells (probably those which yield infectious virus) with nuclear staining in the immunofluorescence test for viral (v) antigen (7, 9).

The present paper reports the transformation by SV40 of cells derived from the liver of a human embryo. Because of a difference in behavior as compared with the known SV40–human cell systems these transformed cells are described in detail.

Materials and Methods. Viruses. SV40, strain VA 45-54, was prepared by serial passage of undiluted stockvirus in BS-C-1 cells and assayed as described previously (1). Sendai virus (10) was obtained from Dr. John F. Enders. It was propagated in the allantoic cavity of 10-day-old embryonated eggs and harvested after 3 days. For fusion experiments, the virus was concentrated and inactivated with propiolactone as described by Neff and Enders (11).

Cell cultures. Cells were prepared from the liver of a human embryo of an estimated 3-months gestation. After one washing in phosphate buffered saline (PBS) the liver was minced, trypsinized, and seeded in 16 × 150-mm tubes. The tubes were incubated at 37° in a stationary position. Three monolayer cultures were inoculated with 0.1 ml of SV40 virus, $10^{6.3}$ $TCID_{50}$/0.1 ml. The BS-C-1 cells were used as indicator cells in cocultivation and fusion experiments.

Media. Cultures were initiated in Eagle's

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