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EFFECT OF ANTI-PARASITE CHEMOTHERAPEUTIC AGENTS ON IMMUNE REACT--ETC(U)
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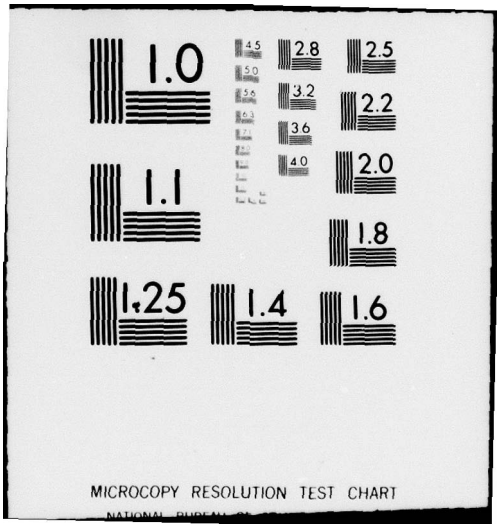
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EFFECT OF ANTI-PARASITE CHEMOTHERAPEUTIC AGENTS ON IMMUNE REACTIONS.

(First Annual Report)

(10) Abdul Ghaffar, PhD

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SUMMARY

Five agents, namely chloroquine phosphate (WR 1544), primaquine phosphate (WR 2975), quinine sulphate (WR 2976-S), quinine dihydrochloride monohydrate (WR 2976-HCl), and 1-1,3-dichloro-6-trifluoromethyl-9-phenanthryl-3-di-(n-butyl)amminopropanol hydrochloride (DTPA) (WR 171669), were studied for their effect on humoral response to sheep erythrocytes (SRBC) as measured by the plaque-forming cell (PFC) contents of spleens, delayed hypersensitivity (DH) reaction to SRBC, and phagocytic activity were studied in mice (see Table 1).

Humoral PFC responses were not affected by WR 1544 or WR 2975. Quinine sulphate (WR 2976-S) and quinine dihydrochloride monohydrate (WR 2976-HCl) suppressed PFC responses when injected one day before, but enhanced these responses when injected one day after the antigen. The DH reactions were not affected significantly by any of the drugs except WR 1544 at the higher dose (100 mg/kg) injected one day post antigen. Phagocytic activity was depressed by the high dose (100 mg/kg) of WR 1544, WR 2975, both low and high doses (40 and 160 mg/kg) of WR 2976-S and by the high dose of compound WR 171669. Deviations from normal controls in these experiments, wherever they occurred, although statistically significant, were never of a tremendously great magnitude and their biological significance might be questionable. Furthermore, the effects of higher doses of these drugs should be considered with caution because these doses were highest which the majority of treated mice could tolerate and survive (see Table 2).

METHODOLOGY

Mice. Male BALB/c mice weighing 20-25 grams were used in all experiments. Mice were housed and maintained in our NIH approved animal facilities.

Antigen and Immunization. Sheep erythrocytes (SRBC) suspended in normal saline were used for immunization. Mice received 0.2 ml of 2.5% (1×10^8) SRBC by the i.p. route for studies involving humoral response, whereas 0.1 ml of 0.25% suspension was injected s.c. on the dorsal side of the neck for immunization for the DH reaction. The challenge for the elicitation of the DH reaction was given as 0.02 ml of 25% suspension intradermally in the right ear.

Assays.

Humoral response: Humoral IgM and IgG responses against sheep cells were measured five days after immunization by assaying spleens for the hemolytic plaque forming cell (PFC) contents by the method described by Cunningham and Szenberg (1).

DH reaction: Mice were injected with 0.02 ml of 25% (1×10^8) SRBC suspension (1×10^8 cells) intradermally in the right ear. Twenty-four hours later, mice were injected i.v. with 2 μCi ^{125}IUdR and 16 hrs later both ears were excised at the hair line and counted in the gamma scintillation spectrometer. The ratio of counts between the SRBC-injected ear and uninjected ear represented the degree of inflammation and indicated the severity of the DH reaction. This method has been described in detail by Vadas et al. (2) and has been proven to be a satisfactory measure of the DH reaction. This method was preferred to foot-pad measurement for the merits of its objectivity.

Phagocytic function: Phagocytic function in mice was measured by the clearance rate of carbon particles as described by Stuart (3). Briefly, mice were injected with 0.2 ml of standardized carbon suspension (OD 0.9 to 1.00 at a dilution of 1/2500 across 1 cm path at 800 nm). Mice were bled at 0, 2, 5, 10, 15 min intervals and a standard volume of heparinized blood obtained. The blood sample was diluted with 0.1% Na_2CO_3 solution and relative amounts of carbon remaining in circulation at various time intervals after injection were estimated in a BL Spectronic-20 photometer at 800 nm. The density readings were converted to a logarithmic scale and plotted against time. The slope of the line indicated the phagocytic index K. The corrected phagocytic index α was calculated as follows:

$$\alpha = \frac{WB}{W\ell + Ws} \times \sqrt[3]{K}$$

where WB was whole body weight of the mouse and $W\ell$ and Ws were liver and spleen weights, respectively.

Drugs and Dosages. Drugs tested and the dosages employed have been indicated in Table 2. All drugs except WR 171669 were dissolved or suspended in normal saline. WR 171669 was dissolved in 20% SMSO, 5% Tween-80, and 75% saline. This solvent was not toxic and gave a colloidal suspension of WR 171669. The solution of WR 2975 was extremely acid and proved very toxic at low pH but the toxicity was reduced when it was neutralized to pH 6.0. At a pH above 6.0, the drug precipitated. WR 1544 and WR 2976-HCl were easily soluble and had a neutral pH. WR 2976-S was insoluble in saline and was injected as a suspension. All drugs were administered in 0.2 ml. Controls recieved the same volume of the solvent.

RESULTS

Humoral Response. Anti-SRBC PFC responses were not significantly altered by WR 1544 and WR 2975. The moderate suppression of IgG PFC by low dose of WR 1544 and by both doses of WR 2975 injected one day after antigen were not statistically significant (see Tables 3 and 4). As indicated from Tables 5 and 6, WR 2976-S and WR 2976-HCl both generally caused slight but significant suppression when injected one day before antigen and caused slight to significant augmentation of anti-SRBC PFC responses.

DH Reactions. Data on the effect of five chemotherapeutic anti-malarial agents have been summarized in Table 7. No statistically significant alteration was observed in the DH reaction of mice treated with WR 2975, WR 2976-S, or WR 2976-HCl, before or after the sensitizing dose of SRBC. WR 1544, at 100 mg/kg dose injected one day after sensitization, however, caused statistically significant depression of the DH reaction.

Phagocytic Function. Table 8 summarizes data on the effect of the five anti-malarial agents on the phagocytic function of mice. It is clear that a high dose of WR 1544 (100 mg/kg) and WR 171669 (100 mg/kg) had significant depressing effects on phagocytic functions of mice when injected before testing. WR 2976-S suppressed phagocytic functions at both doses (i.e., 40 and 160 mg/kg). It is apparent that α values which represent phagocytic indices corrected for spleen, liver, and body weights indicate more marked suppression than the K values of phagocytic indices. This resulted from possible loss of weight in mice treated with these anti-malarial agents. A loss of weight in drug-

treated mice was often noticed, especially when they received the higher dose of certain drugs.

Preliminary Conclusions. Generally, all agents tested to date did not cause a dramatic depression of any of the immune functions. Moderate depression caused by agents WR 2976-S and WR 2976-HCl, although statistically significant, might not be biologically significant since the depression was not as severe as we often observe with known immunosuppressive agents such as cyclophosphamide.

REFERENCES

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TABLE 1

Summary of effects of five anti-malarial agents on immune functions of mice

Drug	Dose (mg/kg)	Anti-SRBC PFC: Drug Pre Ag IgM	Anti-SRBC PFC: Drug Pre Ag IgG	Anti-SRBC PFC: Drug Post Ag IgM	Anti-SRBC PFC: Drug Post Ag IgG	Anti-SRBC DH Reaction: Pre Ag	Anti-SRBC DH Reaction: Post Ag	Phagocytic Activity: K	Phagocytic Activity: α
Chloroquine phosphate (WR 1544)	25	=	=	=	=	=	=	=	=
	100	=	=	=	=	=	↓	=	↓
Primaquine phosphate (WR 2975)	25	=	=	=	=	=	=	↓	=
	100	=?	=?	=	=	=	=	=↓	=
Quinine sulphate (WR 2976-S)	40	↓	↓	↑	↑	=	=	=	↓
	160	↓	=↓	↑	=↑	=	=	=	↓
Quinine dihydrochloride monohydrate (WR 2976-HCl)	50	=	=	↑	=↑	=	=	=	=
	200	=↓	↓	↑	=↑	=	=	=	=
Phenanthryl aminopropanol (WR 171669)	250	=?	=?	↓?	↓?	n.t.	n.t.	=	=
	1000	=?	=?	=?	=?			↓	↓

=, not significantly different from controls
↓, significantly decreased below controls
↑, significantly enhanced above controls
n.t., not tested
=↑ or =↓, differences borderline of significance
?, data not submitted, needs to be confirmed

TABLE 2

Toxicity of five anti-malarial agents studied during this report period, Feb-Aug 1979

Drug	Dose[a]	Mortality within 24 hr	within 2-4 days	within 5-7 days	cumulative %
1544	250	9/10			90
	100[b]	3/65	2/65	2/65	11
	25[b]	0/51	0/51	0/51	0
2975	500	10/10			100
	250	10/10			100
	100[b,c]	7/77	2/77	4/77	17
	25[b]	0/68	0/68	0/68	0
2976-S	1000	5/5			100
	200			2/5	40
	160[b]	0/57	0/57	0/57	0
	40[b]	0/52	0/52	0/52	0
2976 HCl	500	5/5			100
	200[b]	0/31	0/31	1/31	3
	50[b]	0/35	0/35	0/35	0
171669	200	7/10			70
	1500	3/5			60
	1200	0/5	0/5	2/5	40
	1000[b]	0/30	0/30	1/30	3
	250[b]	0/30	0/30	0/30	0

[a] mg/kg

[b] Dose used in studies reported here.

[c] More toxic if used unneutralized at very low pH.

TABLE 3

Effect of primaquine phosphate (2975) on anti-SRBC PFC responses[a]

Drug Time	Treatment Dose	Cells/Spleen x 10^{-6}	PFC per[b] 10^6 IgM	PFC per[b] 10^6 IgG	PFC per[b] Spleen IgM	PFC per[b] Spleen IgG
Pre Ag	Solvent alone		not tested			
	25 mg/kg		not tested			
	100 mg/kg		not tested			
Post Ag	Solvent alone	162.3	2.615 ± 0.033 (412)	3.040 ± 0.040 (1,097)	4.824 ± 0.044 (66,755)	5.362 ± 0.121 (229,946)
	25 mg/kg	129.5[c]	2.659 ± 0.041 (456)	3.026 ± 0.048 (1,061)	4.772 ± 0.051 (59,199)	5.139 ± 0.067 (137,707)
	100 mg/kg	134.1	2.745 ± 0.047[d] (556)	2.985 ± 0.051 (967)	4.863 ± 0.052 (72,899)	5.140 ± 0.065 (138,176)

[a]Drug injected i.p. one day before or one day after 1 x 10^8 SRBC injected i.p. and PFC assays performed five days later.

[b]Log_{10} mean ± 1 s.e. from groups containing a minimum of 8 mice. Numbers in parentheses are geometric means for each group. Data pooled from two experiments.

[c]Statistically significant reduction.

[d]Statistically significant enhancement.

TABLE 4

Effect of chloroquine phosphate (1544) on anti-SRBC PFC responses[a]

Drug Time	Treatment Dose	Cells/Spleen x 10^{-6}	PFC per[b] 10^6 IgM	PFC per[b] 10^6 IgG	PFC per[b] Spleen IgM	PFC per[b] Spleen IgG
Pre Ag	Solvent alone	177.1	2.773 ± 0.039 (593)	2.885 ± 0.080 (767)	4.965 ± 0.068 (97,218)	5.119 ± 0.088 (131,569)
	25 mg/kg	139.7[c]	2.786 ± 0.048 (611)	2.897 ± 0.110 (789)	4.932 ± 0.052 (85,421)	5.043 ± 0.113 (110,359)
	100 mg/kg	158.0	2.804 ± 0.029 (637)	2.928 ± 0.102 (847)	5.003 ± 0.053 (100,764)	5.127 ± 0.127 (133,968)
Post Ag	Solvent alone	162.3	2.615 ± 0.033 (412)	3.040 ± 0.040 (1,097)	4.824 ± 0.044 (66,755)	5.362 ± 0.121 (229,946)
	25 mg/kg	167.3	2.749 ± 0.075 (561)	2.924 ± 0.063 (839)	4.972 ± 0.085 (93,858)	5.147 ± 0.070 (140,236)
	100 mg/kg	161.2	2.647 ± 0.091 (444)	2.943 ± 0.076 (877)	4.865 ± 0.076 (73,310)	5.161 ± 0.091 (144,965)

[a] Drugs injected i.p. one day before or one day after 1 x 10^8 SRBC injected i.p. and PFC assays performed five days later.

[b] Log_{10} mean ± 1 s.e. from groups containing a minimum of 8 mice. Numbers in parentheses are geometric means for each group. Data pooled from two experiments.

[c] Statistically significant reduction.

TABLE 5

Effect of quinine sulphate (2976-S) on anti-SRBC PFC responses[a]

Drug Time	Treatment Dose	Cells/Spleen $\times 10^{-6}$	PFC per[b] 10^6 IgM	PFC per[b] 10^6 IgG	PFC per[b] Spleen IgM	PFC per[b] Spleen IgG
Pre Ag	Solvent alone	123.0	3.146 ± 0.047 (1,398)	3.205 ± 0.084 (1,603)	5.235 ± 0.037 (171,898)	5.315 ± 0.063 (206,572)
	50 mg/kg	115.7	3.001 ± 0.045[c] (1,003)	2.959 ± 0.052[c] (910)	5.064 ± 0.067[c] (115,930)	5.001 ± 0.078[c] (100,212)
	200 mg/kg	92.6[c]	3.059 ± 0.025 (1,147)	3.252 ± 0.102 (1,787)	5.027 ± 0.047[c] (106,490)	5.220 ± 0.107 (165,921)
Post Ag	Solvent alone	170.2	2.701 ± 0.044 (503)	2.979 ± 0.033 (954)	4.932 ± 0.039 (85,604)	5.208 ± 0.034 (161,477)
	50 mg/kg	240.5[d]	2.801 ± 0.060 (632)	2.963 ± 0.073 (919)	5.163 ± 0.050[d] (145,611)	5.336 ± 0.045[d] (217,019)
	200 mg/kg	2.928	2.928 ± 0.041[d] (848)	2.947 ± 0.069 (886)	5.238 ± 0.056[d] (172,917)	5.432 ± 0.087[d] (270,371)

[a] Drugs injected i.p. one day before or one day after 1×10^8 SRBC injected i.p. and PFC assays performed five days later.

[b] Log_{10} mean ± 1 s.e. from groups containing a minimum of 8 mice. Numbers in parentheses are geometric means for each group. Data pooled from two experiments.

[c] Statistically significant suppression.

[d] Statistically significant enhancement.

TABLE 6

Effect of quinine dihydrochloride monohydrate (2976-HCl) on anti-SRBC PFC responses[a]

Drug Treatment Time	Dose	Cells/Spleen $\times 10^{-6}$	PFC per[b] 10^6 IgM	PFC per[b] 10^6 IgG	PFC per[b] Spleen IgM	PFC per[b] Spleen IgG
Pre Ag	Solvent alone	123.0	3.146 ± 0.047 (1,398)	3.205 ± 0.084 (1,603)	5.235 ± 0.037 (171,898)	5.315 ± 0.063 (206,572)
	50 mg/kg	192.6[d]	3.051 ± 0.052 (1,126)	3.131 ± 0.033 (1,351)	5.336 ± 0.048 (216,693)	5.417 ± 0.044 (261,363)
	200 mg/kg	160.0[d]	2.913 ± 0.059[c] (818)	2.622 ± 0.116[c] (419)	5.118 ± 0.066 (131,083)	4.827 ± 0.141[c] (67,091)
Post Ag	Solvent alone	172.8	2.642 ± 0.051 (439)	2.973 ± 0.033 (940)	4.880 ± 0.049 (75,879)	5.211 ± 0.039 (162,491)
	50 mg/kg	208.5	2.752 ± 0.030 (565)	3.017 ± 0.055 (1,040)	5.071 ± 0.033[d] (117,835)	5.336 ± 0.045 (217,019)
	200 mg/kg	209.3	2.931 ± 0.038[d] (852)	3.109 ± 0.057[d] (1,285)	5.253 ± 0.055[d] (178,986)	5.432 ± 0.087 (270,371)

[a]Drugs injected i.p. one day before or one day after 1×10^8 SRBC injected i.p. and PFC assays performed five days later.

[b]Log_{10} mean ± 1 s.e. from groups containing a minimum of 8 mice. Numbers in parentheses are geometric means from each group. Data pooled from two experiments.

[c]Statistically significant suppression.

[d]Statistically significant enhancement.

TABLE 7

Effect of various anti-malarial agents on DH reactions of mice[a]

Treatment		DH Reaction[b]	
Drug	Dose	Drug Pre Ag	Drug Post Ag
	Solvent	1.667 ± 0.238	1.523 ± 0.106
1544	25 mg/kg	2.133 ± 0.250	1.640 ± 0.185
	100 mg/kg	1.953 ± 0.156	0.987 ± 0.048[c]
	Solvent	1.772 ± 0.144	1.513 ± 0.106
2975	25 mg/kg	2.248 ± 0.291	1.815 ± 0.124
	100 mg/kg	1.385 ± 0.151	1.514 ± 0.136
	Solvent	1.451 ± 0.094	1.541 ± 0.114
2976-S	40 mg/kg	1.399 ± 0.187	1.338 ± 0.159
	160 mg/kg	1.131 ± 0.166	1.356 ± 0.102
	Solvent	1.387 ± 0.097	1.633 ± 0.225
2976-HCl	50 mg/kg	1.278 ± 0.116	1.919 ± 0.121
	200 mg/kg	1.595 ± 0.126	2.153 ± 0.153

[a] Drugs injected i.p. one day before or one day after s.c. immunization with 5×10^6 SRBC. Mice challenged intradermally six days later with 1×10^8 SRBC in the right ear and assayed the following day (see Methodology).

[b] Arithmetic means of ratio of radioactivity (cpm) in injected ear over uninjected ear with limits of 1 s.e. Each group contained a minimum of 8 mice.

[c] Statistically significant suppression.

TABLE 8

Effect of various anti-malarial agents on phagocytic function of mice[a]

Treatment		Phagocytic Index[b]	
Drug	Dose	100 x K value	α value
	Solvent	2.699 ± 0.155	4.569 ± 0.215
1544	25 mg/kg	2.864 ± 0.177	4.773 ± 0.299
	100 mg/kg	2.270 ± 0.172	3.794 ± 0.196
	Solvent	2.860 ± 0.170	4.752 ± 0.157
2975	25 mg/kg	2.808 ± 0.158	4.318 ± 0.148
	100 mg/kg	2.649 ± 0.213	4.415 ± 0.187
	Solvent	2.881 ± 0.151	4.784 ± 0.151
2976-S	40 mg/kg	3.121 ± 0.202	3.679 ± 0.196[c]
	160 mg/kg	2.515 ± 0.143	3.749 ± 0.127[c]
	Solvent	2.720 ± 0.135	4.601 ± 0.216
2976-HCl	50 mg/kg	2.627 ± 0.186	4.839 ± 0.111
	200 mg/kg	2.384 ± 0.144	4.474 ± 0.123
	Solvent	2.024 ± 0.139	3.591 ± 0.112
171669	250 mg/kg	1.968 ± 0.245	3.396 ± 0.156
	1000 mg/kg	1.099 ± 0.179[c]	2.974 ± 0.165[c]

[a] Drugs injected i.p. two days before the assay.

[b] Arithmetic mean ± 1 s.e. for groups containing a minimum of 8 mice from two experiments.

[c] Statistically significant depression determined by student's *t* test.