FANSIDAR AND HUMAN LYMPHOCYTE IMMUNE RESPONSE TO PLANT LECTINS

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OBJECTIVE : To investigate the effect of the antimalarial drug Fansidar on human lymphocytes response to plant mitogens and allogeneic cell surface antigens.

BACKGROUND : Fansidar is presently used widely in Thailand as an anti-malarial drug by the National Malaria Eradication Project, the Military, and for self-treatment. Recently, questions have arisen concerning the possible adverse effects on the hematopoietic system that long-term prophylactic use of the drug may have (1, 2).

Recently, a mitogenesis inhibition assay in our laboratory showed atypical results when pooled convalescent sera obtained from patients with falciparum malaria and being treated with Fansidar was added to normal lymphocytes. Normally, sera obtained during the convalescent phase of illness does not inhibit mitogenesis, however pooled convalescent sera from patients treated with Fansidar did inhibit mitogenesis of normal lymphocytes. Since mitogen stimulation has been used as an indicator of general cellular immune responsiveness, the *in vitro* inhibition seen with pooled patient's sera could indicate a drug-induced suppression of :

(1) the immune response of individual recovering from malaria infection, and/or,

(2) general immune competence of lymphocytes from uninfected individuals.

Both components of Fansidar, pyrimethamine and sulfadoxine, have been shown to adversely effect hematopoiesis in humans when the drugs were taken daily for extended periods (1, 2, 3). Thus, it is possible that Fansidar, taken routinely for prolong prophylactic purposes may likewise adversely effect the individual's immune competence.

We are presently using the mitogen inhibition assay to :

(1) confirm that convalescent sera from patients treated with Fansidar (two tablet regimen) inhibit the mitogen stimulation of normal lymphocytes.

(2) determine if chemoprophylaxis with Fansidar can alter the mitogen response of lymphocytes in individuals receiving a single three tablet regimen, or if mitogenesis inhibiting activity is present in sera from individuals on long term (20 weeks) chemoprophylaxis (two tablets every 2 weeks).

METHODS : The effect of pyrimethamine, sulfadoxine or "serum regulatory factors" on the response of test lymphocytes was assessed using a standard mixed lymphocyte culture (MLC) or mitogen stimulation assay (4). Lymphocytes were isolate as previously described (5). Cell viability was assessed by trypan blue dye exclusion to determine drug toxicity against test lymphocytes. Stock solutions of pyrimethamine or sulfadoxine, kindly supplied by Drs. Scholar and Richle (F. Hoffmann-La Roche & Co.), were prepared as follows :

(i) Sulfadoxine 10^{-3} molar (MW 310.3)

250 ml solution		
Sulfadoxine	77.58	mg
NaOH 0.1N ad pH 7.0	2.1	ml
H_2O (ad injectionem)	ad 250	ml

The drug was suspended in a portion of H_2O, then the salt was formed by adding under constant stirring approx. 2.1 ml of 0.1 N NaOH to reach a final pH of 7.0.

The remaining H_2O was added to the final volume of 250 ml; the solution was membrane-filtered (0.2 µm pore size) and autoclaved for 20 minutes at 120°C.

(ii) Pyrimethamine 10^{-4} molar (MW 248.7)

250 ml solution		
Pyrimethamine	6.23	mg
HCl 0.1 N ad pH 4.0	1.03	ml
H_2O (ad injectionem)	ad 250	ml

The drug was suspended in a portion of H_2O, then the salt was formed by adding under constant stirring approx. 1.03 ml of 0.1 N HCl to reach a final pH of 4.0.

The remaining H_2O was added to the final volume of 250 ml; the solution filtered through a 0.2 µm (pore size) membrane filter (e.g., Millipore GS) and autoclaved for 30 minutes at 120°C.

Drug or "serum regulatory factors" effect on *in vitro* assays was assessed by adding media containing either 20% (v/v) control serum and drug or media containing 20% test sera to cultures containing either normal, autologous or allogeneic lymphocytes, then performing the assays as described above. In some cases control sera was obtained from patients treated with quinine.

Results of isotope incorporation are expressed as either stimulation index (SI), calculated as described (1), or as raw counts per minute (cpm).

RESULTS : The results of the *in vitro* pyrimethamine or sulfadoxine drug assays are presented in Table 1. When 10^{-4} molar concentration of pyrimethamine was added to mitogenesis or MLC assays an inhibition of ^{3}H-thymidine uptake of 61.3%, 53.5% and 51.7% was seen in cultures stimulated by PHA, Con A and PWM, respectively. Inhibition of the MLC was 34.7%. No significant inhibition was found in mitogenesis or MLC assays in which sulfadoxine was added. The marked suppression of lymphocyte blastogenesis in cultures containing 10^{-4} molar pyrimethamine was not due to drug toxicity as shown by Table 2.

Sera from individuals receiving Fansidar chemoprophylaxis for up to 20 weeks were collected during weeks 6 and 20. The sera were tested in the mitogenesis and MLC assays to determine if prolong use of the prophylaxis doses of Fansidar alters the response of lymphocyte when assayed with 20% (v/v) sera from these individuals.

Table 3 and Table 4 suggest that sera obtained from Thais during the 6th and 20th week of Fansidar chemoprophylaxis has little effect on the ability of mononuclear cells to respond to mitogen and allogenic cell surface antigen stimulation.

We are presently analysing the raw data to determine if any relationship exists between the level of stimulation of the various cultures and the level of sulfa in the individual sera.

Serum sulfa levels were highly variable and raise the question of the validity in using sulfa drug levels as an indication of Fansidar serum drug levels. This is especially true if serum is not obtained at the same time, post drug administration, from each individual throughout the 20 week test period.

Table 5 shows the effect that pooled patients' sera, collected before and after Fansidar treatment, had on the level of isotope incorporation in mitogen stimulated normal lymphocyte cultures. Acute patients' sera collected before treatment, from both of the groups eventually treated with either quinine or Fansidar, inhibits mitogenesis by normal lymphocytes. This is in agreement with the findings of Wells, et al. (1) in which serum from acutely ill malaria patients (*P.f.* & *P.v.*) added to normal lymphocyte cultures prior to mitogen stimulation inhibits mitogenesis.

When pooled convalescent sera is added to lymphocyte cultures, sera from patients treated with quinine has no mitogenesis inhibitory effect. The SI of cells treated with 28 day sera from quinine treated patients is not significantly different from that of cells receiving media supplement with sera from pooled, uninfected, untreated individuals. However, sera from patients treated with Fansidar obtained 28 days after treatment still had high mitogenesis inhibiting activity to all 3 mitogens tested. Further studies may help to determine if the inhibition is due solely to Fansidar treatment or if the continued inhibition is due to the predisposing malaria infection in combination with Fansidar treatment.

Another experiment designed to see if Fansidar chemoprophylaxis (three tablet therapeutic regimen) altered an individuals' lymphocyte response to

mitogen was recently completed. Lymphocytes were tested from individuals before beginning Fansidar prophylaxis and 14 and 28 days later. Each lymphocyte population was tested in a mitogen inhibition assay on the day of collection against normal, autologous patient, and allogenic patient serum. No consistent, significant differences were seen in any of the tests. However, a larger number of individuals will have to be examined to assess the role of Fansidar and drug-induced suppression of human lymphocytes.

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Table 1. Effect of Sulfadoxine or Pyrimethamine on Mitogen-induced and Mixed Lymphocyte Culture Blastogenic Response of Lymphocytes from Thai Adults.

	CPM ^{3}H-thymidine Uptake*			
	PHA	Con A	PWM	MLC
Sulfadoxine (Molar dose)				
0	31763 ± 10510	34107 ± 10068	26160 ± 9090	2724 ± 1869
10^{-4}	29293 ± 10925	29625 ± 8731	21056 ± 7658	2122 ± 1409
10^{-5}	28521 ± 10817	28727 ± 7025	19816 ± 5439	1976 ± 1701
10^{-6}	30416 ± 18642	27234 ± 9963	18575 ± 6780	2178 ± 1838
10^{-7}	30294 ± 17805	26207 ± 7724	19881 ± 9315	2340 ± 1823
10^{-8}	28900 ± 12812	25660 ± 7551	19647 ± 9715	2309 ± 2129
10^{-9}	30510 ± 17853	33190 ± 10140	25628 ± 9725	2383 ± 1969
Pyrimethamine (Molar dose)				
0	26160 ± 9091	34107 ± 10668	27295 ± 9375	2724 ± 1869
10^{-4}	10169 ± 5737 (61.3%)++	15187 ± 6417 (53.5%)++	13210 ± 6392 (51.7%)++	1781 ± 923 (34.7%)++
10^{-5}	23494 ± 5965	39220 ± 10884	24699 ± 6831	2953 ± 2393
10^{-6}	27388 ± 10981	43000 ± 14152	27113 ± 15406	2842 ± 2256
10^{-7}	23337 ± 12015	33539 ± 10781	21447 ± 9470	2571 ± 2250
10^{-8}	20371 ± 7412	30198 ± 8339	26264 ± 2238	2801 ± 2238
10^{-9}	24484 ± 3416	30357 ± 9346	N.D.**	2297 ± 2018

* Expressed as mean ± standard deviation of 10 sets of experiments. Numerals in parentheses indicate percentage inhibition. +$p < 0.01$, ++$p < 0.05$.

** Not done.

Table 2. Effect of Pyrimethamine and Sulfadoxine on Lymphocyte Viability

	% Viable After 4 Days of Culturing
A + Media	73
A + P(10^{-4})	74.3
A + P(10^{-5})	82.5
A + P(10^{-6})	78.5
A + S(10^{-4})	82
A + S(10^{-5})	88.5
A + S(10^{-6})	86

Table 3. Effect of Sera from Thai Adults Receiving Prolong Fansidar Chemoprophylaxis (1 tablet/week)

CPM ^{3}H-thymidine Uptake*												Sulfa Drug Level (Week)		
PHA (Week)			Con A (Week)			PWM (Week)			MLC (Week)					
1	6	20	1	6	20	1	6	20	1	6	20	1	6	20
27578	19964	38930	30392	14567	23738	20209	9400	20733	-	-	-	0	8.7	11.5
27074	23417	27795	4414	7381	11422	8000	8613	8356	-	-	-	0	0	0
10359	23338	-	7041	9393	-	4157	7016	-	-	-	-	0	N.S.	-
25066	23152	21919	6599	8654	16493	2657	4617	8805	-	-	-	0	0	7.0
11621	22239	8074	4910	7123	2647	6693	8658	5611	402	923	372	7.2	10.3	9.9
21684	13985	17121	18734	15217	11283	4213	890	3437	161	159	363	8.8	10.5	10.8
19106	10068	13733	1275	7137	10077	-	3656	3626	450	-	257	8.2	N.S.	11.5
7058	5928	5548	6205	4462	4219	3472	1596	2440	117	218	73	3.1	4.4	7.6

* Expressed as mean of triplicate culture wells.

Table 4. Effect of Sera from Thai Adults Receiving Prolong Fansidar Chemoprophylaxis (2 tablets/week)

CPM ^{3}H-thymidine Uptake*												Sulfa Drug Level (Week)		
PHA (Week)			Con A (Week)			PWM (Week)			MLC (Week)					
1	6	20	1	6	20	1	6	20	1	6	20	1	6	20
23227	23411	23222	13288	11006	16203	13830	11201	16080	-	-	-	0	N.S.	16.0
17270	26795	-	6960	15669	-	4203	8017	-	-	-	-	N.S.	N.S.	0
19079	20463	-	6851	5986	-	2910	4509	-	-	-	-	0	8.6	N.S.
5861	12475	7954	5019	2886	4752	5656	4535	6777	206	124	460	9.6	9.5	5.5
8984	9827	15313	13407	11837	11749	8802	8274	11177	645	706	440	0	11.2	7.6
13213	8927	17888	13313	15233	6282	4943	8492	3329	712	1081	1773	0	6.9	12.8
15057	14241	21498	1856	1955	2549	6472	4467	5841	1199	464	154	8.9	12.2	6.8
12369	22422	16419	18927	12141	23414	12612	7037	17342	374	257	320	0	13.4	6.6
13595	11499	8520	24386	24339	6705	17549	13763	5180	271	445	3487	6.6	6.1	5.1
11833	9119	11089	6304	4424	7139	2567	1956	2751	494	166	316	9.2	8.9	2.2
8060	7385	19229	3581	5927	15173	2299	2679	6723	242	435	368	10.3	8.8	8.8
13491	9527	11303	5033	9081	17672	4094	2350	4805	122	117	144	8.4	8.6	9.8

* Expressed as mean of triplicate culture wells.

Table 5. Pooled Malaria Patient Serum Effect on Normal Human Lymphocyte Response to Mitogen

Mitogen	Normal Serum	Quinine			Fansidar		
		Day 0[1]	Day 14	Day 28	Day 0	Day 14	Day 28
PHA	156.42[2]	57.84	94.51	107.97	34.24	40.49	27.40
Con A	166.94	56.33	156.53	136.92	24.72	42.35	70.95
PWM	116.06	42.50	114.95	112.05	4.36	7.87	15.67

[1] Pre-treatment serum.

[2] Stimulation index when pooled sera (average 5 patients) is added to wells (20% v/v).