

Fansidar and Human Lymphocyte Immune Response to Plant Lectins

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OBJECTIVE : To determine the effect of Fansidar on the human immune response to selected mitogens.

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 from patients with falciparum malaria who were treated with Fansidar was added to 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 individual 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 : "Serum regulatory factors" and "lymphocytes test populations" will be tested using a standard mitogen stimulation assay (4). Lymphocytes will be isolated from blood using the method of Boyum (5). Serum regulatory factors will be examined by adding media containing 20% (v/v) test sera to cultures containing either normal, autologous, or allogenic lymphocytes, then performing the mitogen stimulation assay described above. Control sera will be obtained from patients treated with quinine. Stimulation index (SI) will be calculated as described (1) and used to measure the non-specific stimulation of lymphocytes as well as the control and test sera's effect on isotope incorporation by normal, mitogen stimulated lymphocytes.

RESULTS : Table 1 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 still had high mitogenesis inhibiting activity 28 days after treatment to all 3 mitogens investigated. 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 with 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.

We are presently using serum from individuals who have been on Fansidar prophylaxis for up to 20 weeks in an effort to determine if prolonged use of prophylaxis doses of Fansidar can lead to the appearance of mitogenesis inhibiting activity.

Testing of the sera from the prophylactic study is in progress

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Table 1. 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
	156.43 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
	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).