

November 7, 1986

Mr. Perry Chapdelaine, Sr.
The Rheumatoid Disease Foundation Route 4, Box 137
Franklin, TN 37064

Dear Perry:

Enclosed please find a summary of our past years work with clotrimazole. And a copy of the Bowman Gray abstract is also included. I am sorry for the delay in sending this out but I have been lecturing almost daily for the past two weeks and for me that is painful and time-consuming. We did receive our final RDF check but as I indicate in the report, I believe that Brian and I are **very** likely to continue with collaborative studies in the future. His observations on the monocyte-lymphocyte interactions are most likely affected by this drug at the level of the cells membrane and therefore may be closely [related] to our *in vitro* observations on phospholipid affects.

We are grateful to have had the support of the Foundation and would be happy to associate with you in the future.

Sincerely yours,

Richard C. Franson, PhD
Associate Professor of Biochemistry

Progress Report: Rheumatoid Disease Foundation/FRANSON
The overall objectives were to

- 1) study mechanism of active of clotrimazole inhibition of SF-PLA₂
- 2) examine its effects on other PLA₂s
- 3) determine the levels of PMN-PLA₂ from patients ± clotrimazole according to the Bowman Gray/Dr. Turner clinical trials
- 4) study effect of related imidazole compounds
- 5) look for endogenous inhibitors of PLA₂ in synovial fluid.

Results

1,2,4: We have demonstrated clearly that clotrimazole inhibits the human synovial fluid PLA₂ (as well as other neutral-active and Ca²⁺-dependent PLA₂s) in a Ca²⁺-dependent fashion. That is, at lower and more physiologic levels of Ca²⁺ (10-1000µM) clotrimazole produced dose-dependent inhibition. Because membrane phospholipids contain the bulk of arachidonate (the precursor for prostanoids and leukotrienes) in the SN-2 position of the molecule, the ability of this molecule to act as an anti-inflammatory agent was proposed. Tinidazole and histamine had little or no effect on enzyme activity, similar results were obtained with metronidazole (Flagyl). The mode of action appears to binding of clotrimazole to the phospholipid substrate since centrifugation studies of drug + substrate *E. coli* resulted in cosedimentation of both components leaving no inhibiting activity in the supernatant fraction.

3: In January, 1986 we began the analysis of human PMN's derived from the patients at Boman Gray who entered the clinical trial. Below is a list of the results patient by patient which was shipped to Dr. Duane Smith at Bowman Gray and which served as the basis for the *in vitro* studies discussed in the ⁴ abstract.

Patient	Date	SA (pmols/hr/10 ⁷ cell equiv.)
1 a		
b	01/24/86	106.8

		pt dropped	
2			
3	a	12/23/85	83.8
	b	03/17/86	59.0
4	a	01/22/86	55.2
	b	04/16/86	48.8
5	a	02/07/86	74.4
	b	05/02/86	12.8
6	a	02/14/86	14.1
	b	03/14/86	53.9
7	a	02/14/86	41.5
	b	05/07/86	84.7
8	a	03/06/86	53.9
	b	05/29/86	39.8
9	a	03/17/86	55.2
	b	06/16/86	48.8
10	a	04/07/86	52.6
	b	06/30/86	21.8
11	a	_____	_____
	b	05/14/86	26.9

Judging from the abstract, it is a partial correlation that can not stand up to rigorous statistical analysis.

5) In this area we are continuing the search for endogenous regulators of what we believe is a proinflammatory PLA₂ in SF. It is clear from these studies that both inhibitory lipids and proteins are present in synovial fluid that moderate the expression of PLA₂. We believe that clotrimazole is an additional modulator and that the very interesting studies that Dr. Susskind now pursues with respect to the drugs' affect on monocytes may be membrane-lipid mediated and thus be directly related to our basic observation of phospholipase inhibition. In this area Dr. Susskind and I are very likely to collaboration in the future.

Respectfully Submitted,

Richard C. Franson
Associate Professor of Biochemistry