Medical College of Virginia
Virginia Commonwealth University

June 7, 1985

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

Dear Mr. Chapelaine:

Enclosed please find a grant application-request to the Rheumatoid Disease Foundation entitled " The Effect of Clotrimazole and Related Agents on Cell-free, Calcium-dependent Phospholipase A_2 Activity in Human Synovial Fluid". We believe that this work represents an exciting step forward in understanding and perhaps intervening in the inflammation associated with active arthritic disease. We hope that the Foundation feels similarly.

Sincerely yours,
Richard C. Franson

Richard C. Franson, PhD
Associate Professor of Biochemistry

Department of Biochemistry • P.O. Box 614 • Richmond, Virginia 23298-0001 • (804)786-9762

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RHEUMATOID DISEASE FOUNDATION GRANT REQUEST:

Effect of Clotrimazole and Related Agents on Cell-free, Calcium-dependent Phospholipase A_2 Activity in Human Synovial Fluid

Support Requested: from July 1, 1985 through June 30,1986

Itemized Budget:

technical salary + fringe benefits	$ 18,200
chemicals, radioisotopes & glassware	$ 2,500
	$ 20,700

Rationale and Objectives

We have found that phospholipase A_2 (PLA_2) activity, an enzyme system involved in inflammation and corticosteriod action, is elevated in the synovial fluid of patients with active arthritic diseases. Based on this observation, we have focused our studies in recent years on the occurrence and regulation of Ca^{2+}-dependent and neutral-active PLA_2s in human synovial fluid and human polymorphonuclear leukocytes.

Despite a lack of funding for this project, we have examined the synovial fluid from over 50 patients with a variety of arthritic diseases. As the accompanying reprint describes, we have found a potent Ca^{2+}-dependent PLA_2 activity in human synovial fluid. The enzyme is cell-free in this fluid and is directly inhibited by some, but not all, nonsteroidal anti-inflammatory agents.

This PLA_2 enzyme system is also of general interest to the study of inflammation because one of the known mechanisms of action of steriods is the induction (by protein synthesis) of an anti-PLA_2 protein, referred to as lipomodulin or macrocortin. Thus, it is believed that PLA_2s are regulated in situ by endogenous as well as exogenous agents.

Clotrimazole: As an Antagonist of PLA_2s

Recently, at the suggestion of Dr. William Regelson, we tested the anti-fungal drug, clotrimazole for its ability to influence the human synovial fluid PLA_2. This drug, an imidazole derivative, has received recent attention because of its anti-inflammatory activity in patients with arthritis, raising the possibility that either the etiologic agents producing this inflammatory disease could be amebal or fungal, or that a more general mechanism is involved. In this regard, and of importance to our work, immunologic studies now indicate that antigens of the pathogenic ameba, Naegleria fowleri are present in human synovial fluid, and in past work (see enclosed reprints) we have demonstrated that during growth, these ameba secrete large quantities of phospholipases spontaneously, and that these cell-free enzymes degrade the phospholipids of human myelin suggesting a possible relationship to multiple sclerosis.

Our preliminary studies with clotrimazole are shown in figure 1 (page 5): clotrimazole produces dose-dependent inhibition of the synovial fluid PLA_2. It is particularly interesting that the inhibition is inversely related to the concentration of added $CaCl_2$. Thus, no inhibition is noted with 5mM added $CaCl_2$, a concentration typically used for the in vitro assay of PLA_2 activity. As the concentration of added $CaCl_2$ decreases to more physiologic levels (10-100 uM) clotrimazole produces dose-dependent inhibition with an IC_{50} in the micromolar range.

In summary, these preliminary studies indicate that clotrimazole has direct inhibitory effects on human synovial fluid PLA_2 activity and the data suggests that this inhibition may constitute a direct mechanism for the anti-inflammatory or anti-rheumatoid arthritic activity of this drug.

Discussion

A main feature of inflammation and cell injury in arthritis is thought to involve the generation of leukotrienes and prostaglandins as a result of mobilization of the fatty acid precursor, arachidonate, from membrane phospholipid. Arachidonate does not exist as a free fatty acid to any appeciable extent in biological systems, and it is predominantly esterified in the 2-position of mammalian phospholipids. Therefore, arachidonate must be released from membrane phospholipid in response to various stimuli by the activation of phospholipases. Two pathways are thought to contribute to mobilization of arachidonate in mammalian systems: direct deacylation of the 2-position of phospholipids by a phospholipase A_2 (PLA_2) and the concerted action of a phosphatidylinositol-specific phospholipase C (PI-PLC) and a diglyceride lipase. Because our preliminary studies indicate that large quantities of PLA_2 activity are found in human synovial fluid with little or no PI-PLC activity, the above observations are pertinent to our area of interest and hopefully those of the Rheumatoid Disease Foundation. Thus, we have shown that

1) Naegleria fowleri is a potent producer of phospholipase A_2.
2) phospholipase A_2 activity is elevated during active joint inflammation in the synovial fluid of rheumatoid arthritic joints.
3) clotrimazole, an anti-amebic and anti-fungal agent thought to induce remission in rheumatoid arthritis, is a potent inhibitor of phospholipase A_2.

Request: In view of the above, we request support from the Rheumatoid Disease Foundation to:

1) carefully study the mechanism of action of clotrimazole on the inhibition of the human synovial fluid PLA_2
2) examine the effects of this drug on other phospholipases associated with human PMNs (polymorphonuclear leukocytes)

3) determine the levels of PLA_2 in synovial neutrophils and if possible in synovial fluid from patients treated with clotrimazole (cooperative effort with Dr. Turner at Bowman Gray, Winston-Salem, NC)

4) study the effect of other imidazole compounds, such as histamine, on synovial fluid PLA_2

5) examine human synovial fluid for the presence of endogenous components that share anti-PLA_2 and anti-inflammatory activities, and

6) collaborate with Dr. Susskind (Department of Surgery, MCV) in his Rheumatoid Disease Foundation study attempting to isolate Naegleria or other infectious organisms from rheumatoid synovia.

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Richard C. Franson 6/5/85

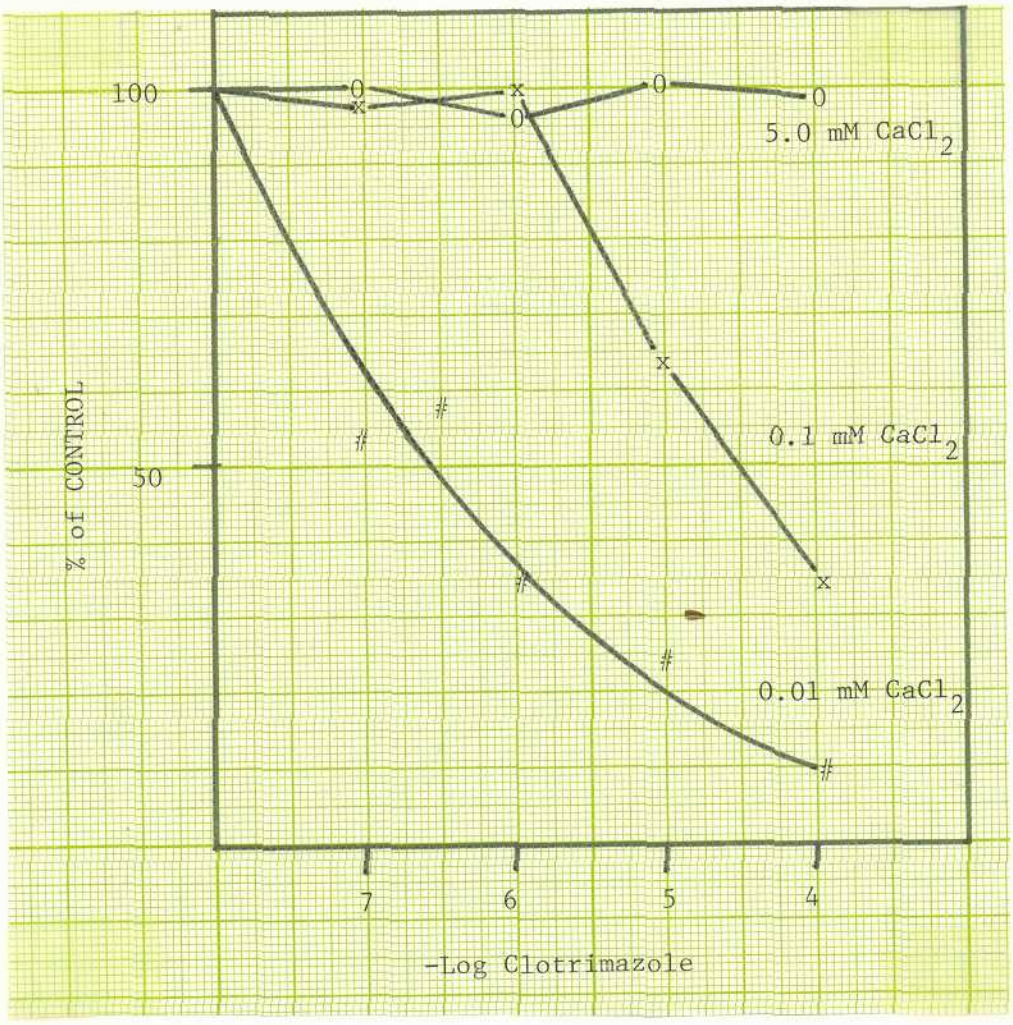

Figure 1

Inhibition of Human Synovial Fluid Phospholipase A_2 Activity by Clotrimazole as a Function of Added $CaCl_2$

CURRICULUM VITAE

1. PERSONAL INFORMATION

NAME: Richard C. Franson

DATE OF BIRTH: 12/14/43

PLACE OF BIRTH: Woburn, Massachusetts

CITIZENSHIP: U.S.A.

SOCIAL SECURITY NUMBER: 016-34-3082

MARITAL STATUS: Married, three children

HOME ADDRESS: 3010 Comet Road, Richmond, VA 23229

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2. EDUCATION

Bowman Gray School of Medicine, Winston-Salem, NC
1972 Ph.D. Biochemistry under the supervision
1970 M.S. Biochemistry of Dr. Moseley Waite
University of Massachusetts, Amherst, MA
1965 B.S. Botany

3. MILITARY SERVICE RECORD

1966-68 Hospital Corpsman - Clinical Chemist, U. S. Navy

4. POSTDOCTORAL TRAINING or SPECIAL EXPERIENCE

1979-80 Educational leave with Professors H. van den Bosch and L.L.M. van Deenen, Laboratory of Biochemistry, University of Utrecht, The Netherlands
1972-74 U.S.P.H.S. Postdoctoral Fellow, New York University Medical Center under the supervision of Dr. Peter Elsbach

5. ACADEMIC APPOINTMENTS - WORK EXPERIENCE

Associate Professor of Biochemistry, Medical College of Virginia, 4/82 - present
Associate Professor of Biophysics and Biochemistry, Medical College of Virginia, 7/79 - 4/82

Assistant Professor of Biophysics and Biochemistry, Medical College of Virginia, 9/77 - 7/79
Assistant Professor of Biophysics, Medical College of Virginia, 9/75-9/77
Instructor of Experimental Medicine, N.Y.U. School of Medicine, 9/74-9/75

6. MEMBERSHIPS - SCIENTIFIC, HONORARY & PROFESSIONAL SOCIETIES

Sigma Xi, Harvey Society, American Association for the Advancement of Science, American Heart Association, New York Academy of Sciences, The Biophysical Society, American Society of Biological Chemists

7. HONORS - NIH Research Career Development Award 1980-84
NIH Young Investigator Award 1976-79

8. BIBLIOGRAPHY

A. Papers Published: Richard C. Franson, Ph.D.

1. Franson, R., Waite, M. and LaVia, M. Identification of phospholipases A_1 and A_2 in the soluble fraction of rat liver lysosomes. Biochemistry 10:1942, 1971.

2. Franson, R., Waite, M. and Weglicki, W. Phospholipase A activity of lysosomes of rat myocardial tissue. Biochemistry 11:472, 1972.

3. Wang, P., Franson, R., DeChatelet, L., Waite, M. and McCall, C. Effect of methylprednisolone on some BCG-mediated changes in rabbit alveolar macrophages. Infec. Immun. 6:982, 1972.

4. Franson, R. and Waite, M. Lysosomal phospholipases A_1 and A_2 of normal and Bacillus Calmette Guerin-induced alveolar macrophages. J. Cell. Biol. 56:621, 1973.

5. Franson, R., Beckerdite, S., Wang, P., Waite, M. and Elsbach, P. Some properties of phospholipases of alveolar macrophages. Biochim. Biophys. Acta, 296:365, 1973.

6. Elsbach, P., Pettis, P., Beckerdite, S. and Franson, R. Effects of phagocytosis by rabbit granulocytes on macromolecular synthesis and degradation in different species of bacteria. J. Bacteriol. 115:396, 1974.

7. Franson, R., Patriarca, P. and Elsbach, P. Phospholipid metabolism by phagocytic cells. Acid and alkaline phospholipases A associated with rabbit polymorphonuclear leukocyte granules. J. Lipid Res. 15:380, 1974.

8. Beckerdite, S., Mooney, C., Weiss, J., Franson, R. and Elsbach, P. Early and discrete changes in permeability of Escherichia coli and certain other gram-negative bacteria during killing by granulocytes. J. Exp. Med. 140:396, 1974.

9. Elsbach P., Beckerdite, S., Pettis, P. and Franson, R. Persistence of regulation of macromolecular synthesis by E. coli during killing by disrupted granulocytes. Infec. Immun. 9:663, 1974.

10. Weiss, J., Franson, R., Beckerdite, S. and Elsbach, P. Partial characterization and purification of a rabbit granulocyte factor that increases permeability of E. coli. J. Clin. Invest. 55:33, 1975.

11. Weiss, J., Franson, R. and Elsbach, P. Granulocyte phospholipase A-induced permeability alterations in E. coli. Biochim. Biophys. Acta. 436:154, 1976.

12. Laychock, S., Franson, R., Weglicki, W. and Rubin, R. Identification and partial characterization of phospholipases in isolated adrenocortical cells. Biochem. J. 164:753, 1977.

13. Franson, R., Weiss, J., Martin, L., Spitznagel, J. and Elsbach, P. Phospholipase A activity associated with the membranes of human polymorphonuclear leukocytes. Biochem. J. 167:839, 1977.

14. Franson, R., Dobrow, R., Weiss, Jr., Elsbach, P. and Weglicki, W. Isolation and characterization of a phospholipase A_2 from an inflammatory exudate. J. Lipid Res. 19:18, 1978.

15. Franson, R., Pang, D., Towle, D. and Weglicki, W. Phospholipase A activity of highly enriched preparation of cardiac sarcolemma from hamster and dog. J. Mol. Cell. Cardiol. 10:921, 1978.

16. Franson, R. and Waite, M. The relation between calcium requirement, substrate charge and rabbit polymorphonuclear leukocyte phospholipase A_2 activity. Biochemistry 17:4029, 1978.

17. Franson, R., Pang, D. and Weglicki, W. Modulation of lipolytic activity in isolated canine cardiac sarcolemma by isoproterenol and propranolol. Biochem. Biophys. Res. Commun. 90:956, 1979.

18. Elsbach, P., Weiss, J., Franson, R., Beckerdite-Quagliata, S., Schneider, A., Harris, L. Separation and purification of a potent bactericidal/permeability increasing protein and a closely associated phospholipase A_2 from rabbit polymorphonuclear leukocytes. J. Biol. Chem. 254:11,000, 1979.

19. Jesse, R. and Franson, R. Modulation of purified phospholipase A_2 activity from human platelets by calcium and indomethacin. Biochim. Biophys. Acta 575:467, 1979.

20. Schrey, M., Franson, R. and Rubin, R. Further characterization of a calcium activated phospholipase A_2 in cat adrenal cortex. Cell Calcium, 1:91, 1980.

21. Franson, R., Eisen, D., Jesse, R. and Lanni, C. Inhibition of highly purified mammalian phospholipases A_2 by nonsteroidal anti-inflammatory agents: modulation by calcium ions. Biochem. J. 186:633, 1980.

22. Evans, H., Franson, R., Qureshi, G.D., Moo-Penn, W.F.: Isolation of anti-coagulant proteins from cobra venom (Naja Nigricollis): identity with phospholipase A_2. J. Biol. Chem. 255:3793, 1980.

23. Owens, K., Pang, D., Franson, R. and Weglicki, W. Lipids in myocardial membranes: susceptibility of a fraction enriched in sarcolemma to hydrolysis by an exogenous mammalian phospholipase A_2. Lipids 15:534, 1980.

24. Lanni, C. and Franson, R. Localization and partial purification of a neutral-active phospholipase A_2 from BCG-induced rabbit alveolar macrophages. Biochim. Biophys. Acta 658:54, 1981.

25. Franson, R. and van den Bosch, H. Lysophospholipase activity of bovine adrenal medulla: a re-evaluation. Biochim. Biophys. Acta 711:75, 1982.

26. Hysmith, R. and Franson, R. Elevated Levels of Cellular and Extracellular Phospholipases from Pathogenic Naegleria fowleri. Biochim. Biophys. Acta 711:26, 1982.

27. Franson, R. and Weir, D. Isolation and Characterization of a Membrane-Associated, Calcium-Dependent Phospholipase A_2 from Rabbit Lung. Lung 160:275, 1982.

28. Hysmith, R. and Franson, R. Degradation of Human Myelin Phospholipids by Phospholipase-Enriched Culture Media of Pathogenic Naegleria fowleri. Biochim. Biophys. Acta 712:698, 1982.

29. Franson, R., Weir, D. and Thakkar, J. Solubilization and Characterization of a Neutral-Active, Calcium-Dependent Phospholipase A_2 from Rabbit Heart and Isolated Chick Embryo Myocytes. J. Mol. Cell. Cardiol. 15:189, 1983.

30. Thakkar, J., Sperelakis, N., Pang, D. and Franson, R. Characterization of Phospholipase A_2 Activity in Rat Aorta Smooth Muscle Cells. Biochim. Biophys. Acta 750:134, 1983.

31. Namba, M., Suga, M., Dannenberg, A., Hastie, A. and Franson, R. Immunocytochemical Demonstration of Rabbit Ribonuclease, Lysozyme and Phospholipase A_2 in Pulmonary Alveolar Macrophages. J. Retic. Endo. Soc., 34: 425, 1983. J. Reticulo. Endo. Soc. 34:425, 1983.

32. Thakkar, J., East, J., Seyler, D. and Franson, R. Characterization of a surface-active phospholipase A_2 in mouse spermatozoa. Biochim. Biophys. Acta 754:44, 1983.

33. Rosenblum, W.I., Hirsch, P.D. and Franson, R. Overnight food deprivation in normal and diabetic mice markedly enhances thromboxane production by arachidonate stimulated platelet rich plasma and markedly increases platelet phospholipase A_2 activity. Prost. & Med. 31:557, 1983.

34. Bartolf, M. and Franson, R. pH-Dependent Modulation of Phospholipase A_2 Activity by Alkaline Cations and Catecholamines in a Granule-enriched Fraction of Adrenal Medulla. Biochim. Biophys. Acta, 793:379, 1984

35. Franson, R., Evans, H., Thakkar, J. and Sperelakis, N. Inhibition of human platelet aggregation and calcium-dependent phospholipase A_2 activity by calcium antagonists: Evidence for intracellular effects of calcium slow channel blockers. In: Calcium Antagonists, M. Sperelakis, ed., pp. 327-338, Boston, 1984.

36. Eisen, D., Bartolf, M. and Franson, R. Inhibition of lysosomal phospholipases C and A in rabbit alveolar macrophages, PMN-leukocytes, and rat liver by sodium bisulfite. Biochim. Biophys. Acta, 793: 10, 1984.

37. Kyger, E. and Franson, R. Nonspecific inhibition of enzymes by p-bromophenacylbromide: inhibition of human platelet phospholipase C and modification of sulfhydryl groups. Biochim. Biophys. Acta, 794:96, 1984

38. Franson, R., Kyger, E. and Weir, D. Pharmacologic modulation of calcium-dependent phospholipase A_2 activity isolated from rabbit lung. Prog. Resp. Res., 18:176, 1984.

39. Thakkar, J., East, J. and Franson, R. Modulation of phospholipase A_2 activity associated with human sperm membranes by divalent cations and calcium antagonists. Biol. Reprod., 30: 679, 1984.

40. Kramer, C., Franson, R.C. and Rubin, R. Regulation of calcium metabolism, phosphatidylinositol turnover, and enzyme secretion by phorbol dibutyrate in rabbit neutrophils. Lipids, 19:315, 1984.

41. Franson, R.C. and Rosenblum, W. Diglyceride lypase activity in mouse, rat and human platelets. Throm. Res., 36:323, 1984.

42. Ferragut, J., Gonzalez-Ros, J., Peterson, D., Weir, D., Franson, R.C. and Martinez-Carrion, M. Rapid purification of a phospholipase-free α-bungarotoxin: Maintenance of carrier barriers of acetylcholine receptor membranes upon preincubation with purified toxins. Arch. Biochem. Biophys., 235:628, 1984.

43. Franson, R.C., Blackwell, W., Eisen, D. and Hess, M. Cardiac sarcoplasmic reticulum dysfunction during global ischemia: Phospholipid alterations induced by lysosomal sphingomyelinase-phospholipase C and lysosomal stabilization by pretreatment with superoxide dismutase and catalase. Circ. Res., in press, 1985.

44. Creutz, C., Dowling, L., Kyger, E. and Franson, R. Phosphatidylinositol-specific Phospholipase C Activity of Chromaffin Granule-binding Proteins. J. Biol. Chem., in press, 1985.

45. Kyger, E. and Franson, R. The In Vitro Effects of Cations on Human Platelet Phosphatidylinositol-specific Phospholipase C. Arch. Biochem. Biophys., submitted, 1985.

46. Bartolf, M. and Franson, R. Characterization and Localization of a Neutral-Active Mn^{2+} -Mg^{2+}-dependent Sphingomyelinase in Bovine Adrenal Medulla. Lipids: submitted, 1985.

B. Chapters

1. Waite, M., Griffin, H. and Franson, R. (1976) Lysosomal phospholipases A. In: Lysosomes in Biology and Pathology (Dingle, J.T. and Fell, H.B., eds.) vol. 6, pp. 257-305, North Holland Publishing Company, London.

2. Waite, M., Rao, R., Griffin, H., Franson, R., Miller, C., Sisson, P., and Frye, J. (1981). Phospholipases A_2 from lysosomes and plasma membranes of rat liver. In: Methods in Enzymology (Lowenstein, J. ed.) vol. 71, pp 674-690. Academic Press, New York.

3. Franson, R. (1981) Intracellular metabolism of ingested phospholipid. In: Research Monographs in Cell and Tissue Physiology (Knight, G. ed) chapter 12, pp 347-378, North Holland Publishing Company, Amsterdam.