

The Bowman Gray
School of Medicine

Department of Clinics
Rheumatology

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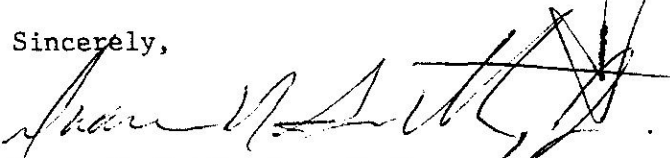
Dr. Paul K. Pybus
404 United Building
181 Church Street
Peitermaritzburg 3201
Republic of South Africa

Dear Dr. Pybus:

It was a pleasure meeting with you on July 7th and discussing my research. I had hoped on getting back to you earlier to discuss the final results; however, I have been trying to enter the data into the computer in an effort to begin examining if any correlations exist with the clinical state of the patients undergoing the Clotrimazole study. Currently this work is still in progress. So far, the results of these patients can only be examined at the start of the study while they are on their various non-steroidal anti-inflammatory drugs. We really won't know how well they did on the Clotrimazole until they all have finished and the code is finally broken. However, I have also recently been in touch with Dr. Richard Franson concerning his cell-free phospholipase assays in neutrophils from these same patients and we are currently working on correlating the results between the two studies. I am also enclosing on a separate page a brief explanation at how I arrived at the results of my study.

Again, it was a pleasure meeting you. I hope you are feeling better and I am sorry for the delay. I am sending two copies of these results to you, one in South Africa and one in Franklin, TN, whichever will get to you first.

Sincerely,


Duane M. Smith, Jr., Ph.D.

DMS/mam

cc: Dr. Robert Turner

Enclosure

NO
ID

R-11208 CLOTRIMAZOLE VS PLACEBO
 TWELVE WEEK DOUBLE BLIND STUDY
 ARACHIDONIC ACID METABOLISM
 FROM PERIPHERAL BLOOD NEUTROPHILS

NAME	PT #	CODE	DATE	VISIT	NSAID	CE/TB	FFA	DS/HETE	S-HETE	MG/LTB4	PE	PS/PI	PC	SPHY/LPG
ROBERTS, DONNA	1	ROB	06-Dec-85	A	NAPROSYN	0.4%	0.2%	0.8%	3.4%	3.8%	-0.1%	-4.6%	-3.8%	-0.1%
CUZZONE, ROBERT	2	CUZ	13-Dec-85	A	INDOCIN	2.8%	-0.1%	1.2%	3.3%	4.7%	0.9%	-0.3%	-3.8%	-0.5%
YONCE, JOHN	3	YON	23-Dec-85	A	DISALCID	-3.2%	5.3%	2.6%	0.0%	7.0%	-1.9%	-12.5%	-6.7%	0.3%
LEONARD, HERBERT	4	LED	22-Jan-86	A	NAPROSYN	1.9%	2.2%	2.4%	6.1%	5.0%	0.6%	-14.4%	-3.5%	-0.3%
WILES, NAOMI	5	WIL	07-Feb-86	A	NAPROSYN	2.0%	0.2%	0.7%	2.0%	1.8%	-0.6%	-4.6%	-1.8%	0.3%
JONES, ALMA	6	JON	14-Feb-86	A	NAPROSYN	-3.0%	-0.5%	1.5%	0.1%	2.5%	2.5%	-1.8%	-0.7%	-0.6%
CASON, FRANK	7	CAS	14-Feb-86	A	ASPIRIN	-4.7%	4.1%	2.1%	3.1%	5.9%	-0.2%	-6.3%	-4.0%	0.1%
DAVIS, MARY	8	DAV	06-Mar-86	A	NAPROSYN	3.0%	0.9%	1.6%	1.9%	3.5%	0.5%	-8.6%	-2.9%	0.1%
ALDERFER, FLORENCE	9	ALD	17-Mar-86	A	TOLECTIN	0.9%	3.0%	2.2%	5.2%	4.0%	-0.7%	-11.2%	-3.6%	0.1%
LINVILLE, WILLIAM	10	LIN	07-Apr-86	A	DOLBID	1.6%	1.5%	1.1%	2.0%	3.8%	-0.3%	-4.8%	-4.7%	-0.5%
ROBERTS, DONNA	1	ROB	24-Jan-86	B	NAPROSYN	0.1%	5.9%	5.1%	7.7%	6.9%	-1.0%	-17.9%	-6.8%	0.0%
YONCE, JOHN	3	YON	17-Mar-86	B	DISALCID	-2.7%	4.9%	2.9%	6.8%	6.0%	-0.4%	-11.6%	-6.1%	0.3%
LEONARD, HERBERT	4	LED	16-Apr-86	B	NAPROSYN	-0.9%	1.5%	1.4%	2.2%	8.4%	0.0%	-8.8%	-3.6%	-0.3%
WILES, NAOMI	5	WIL	02-May-86	B	NAPROSYN	-5.0%	1.6%	1.9%	2.8%	5.3%	0.0%	-3.9%	-2.6%	0.1%
JONES, ALMA	6	JON	14-Mar-86	B	NAPROSYN	0.1%	-0.3%	1.0%	2.6%	3.7%	0.3%	-4.9%	-2.3%	-0.1%
CASON, FRANK	7	CAS	07-May-86	B	ASPIRIN	-1.6%	1.5%	-0.4%	0.6%	4.2%	-0.3%	-3.0%	-1.1%	-0.2%
DAVIS, MARY	8	DAV	29-May-86	B	NAPROSYN	-2.0%	-0.4%	1.0%	1.2%	2.8%	0.4%	-1.3%	-0.3%	-1.3%
ALDERFER, FLORENCE	9	ALD	16-Jun-86	B	TOLECTIN	0.3%	0.1%	0.3%	1.6%	4.5%	-0.9%	-3.5%	-1.8%	-0.5%
LINVILLE, WILLIAM	10	LIN	30-Jun-86	B	DOLBID	2.4%	2.0%	-0.8%	1.9%	2.5%	0.0%	-6.0%	-1.8%	-0.1%
ABERNATHY, JOHN	11	ABE	14-May-86	B	ASA-DOLB	1.4%	2.7%	1.5%	6.8%	10.2%	-1.2%	-14.4%	-6.2%	-0.7%

R-1120A Clotrimazole vs. Placebo
Twelve week double blind study
Arachidonic acid metabolism from peripheral blood neutrophils

Research Protocol

Briefly, human neutrophils were isolated from whole heparinized blood following plasma gel sedimentation of erythrocytes, lymphoprep gradient removal of blood monocytes, and a brief hypotonic lysis to remove contaminating erythrocytes. Generally this procedure yields 95% neutrophils with greater than 95% viability. Neutrophils at 3.5×10^7 neutrophils/ml PBS are prelabeled with $0.2 \mu\text{M}$ [^3H] arachidonic acid (0.5u Ci) for two hours at 37° . The cells are then washed to remove any unbound label and then resuspended to 3.5×10^7 neutrophils/ml PBS buffer. The cells are then split into 2 (0.5 ml) aliquots and stimulated with either 0.5 ml PBS (control) or 0.5 ml A23187 ($10 \mu\text{M}$ final) for five minutes at 37° . The reaction is stopped and the lipids are extracted. The lipids are then separated into different classes by thin layer chromatography. The various lipids are isolated and the radioactivity in each determined by liquid scintillation. The results then can be expressed several ways: they can be expressed as the difference in actual counts in each lipid species between A23187 stimulated and control (PBS) treated cells, i.e., [^3H] FFA cpm (A23187-stimulated) - [^3H] FFA cpm (PBS) = difference in [^3H] FFA cpm or usually a more consistent procedure and the procedure employed here (thus eliminating differences in sample size or extraction efficiencies) is to express the radioactivity for each separated lipid as a percentage for that lipid relative to the total recovered lipids for that aliquot of cells. Results are then based on the differences in the percentage distribution of the radiolabel in a particular lipid between A23187 stimulation and control (PBS) cells. Therefore a positive number indicates an increase distribution at the radiolabel into these lipid species upon stimulation, whereas a negative number indicates a relative decrease in the distribution of the radiolabel in these lipid species, thus showing where the label is coming from and where it is going or being metabolized.