

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 4/1/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 6,317
PR 10406 - 31 Mar 88

Attachment

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Progress Report for PSTC Project

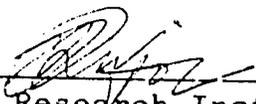
Titled

"Evaluation of Polyamine
Synthesis Inhibition as Therapy for
Trypanosoma rhodesiense Infection"

For the period 1 October 1986 - 31 March 1988

Project Identification Number: 6.317

Principal Investigator:

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Co-Principal Investigators:

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Assistant Professor

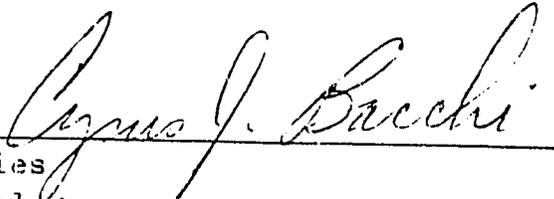
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APR 1 -

There have been delays due to technical problems with release of funds from AID to KETRI. These have been primarily caused by unexplained poor mail connections between Muguga, Kenya, AID, Nairobi, Kenya and AID, Washington, D.C. This problem has been circumvented in a clumsy but effective manner by relaying documents from KETRI via DHL courier service to Dr. Clarkson in New York who then sends them on to the AID office in Washington, DC. As yet no funds have been received by KETRI for the project. In spite of this Dr. Njogu has used some of the very limited KETRI funds available to start the project and work has proceeded as best as possible. This problem in funding has seriously delayed some aspects of the work but substantial progress has been made in spite of this.

The poor communication between KETRI in Muguga, Kenya and AID in Washington, DC has also resulted in a lack of filing of progress reports in the past but this will not occur in the future.

Synopsis:

The most important work done by this project to date is the development of methods to study the pharmacokinetics of DFMO in primates and the discovery of a spectrum of DFMO sensitivity among various strains of T. b. rhodesiense. Pharmacokinetic studies are important because they will allow the development and testing of optimal DFMO dosage in a non-human primate. Observation of a spectrum of sensitivity to T. b. rhodesiense is important because it reflects what has been observed in clinical use of DFMO against T. b. rhodesiense infection in humans. The ability to reproduce the spectrum in the laboratory will allow the development of means to overcome refractoriness to DFMO - most probably by combination with other drugs.

Major Goals:

- 1) Establish working relationships among KETRI, New York University and Pace University:
- 2) Establish tissue culture laboratory at KETRI
- 3) Examine KETRI strains of Trypanosoma brucei rhodesiense for sensitivity to DFMO in vitro and in vivo to determine the basis of T. b. rhodesiense refractoriness to DFMO.
- 4) Test the ability of combinations of other drugs with DFMO to overcome the refractoriness of T. b. rhodesiense to DFMO.
- 4) Examine ornithine decarboxylase (ODC) activities, uptake of radiolabelled difluoromethylornithine (DFMO), and other biochemical parameters in T. b. rhodesiense strains.
- 5) Begin studies with primates on the pharmacokinetics of DFMO.
- 6) Establish the basis for a staff-training program between KETRI and New York University.

Progress Towards Reaching the Goals:

Working relationship with KETRI: During the first year of the grant, Drs. Clarkson and Bacchi each spent 5 weeks at KETRI. The following activities were undertaken: establishment of a limited-capacity biochemistry laboratory; training Mr. Henry Waithaka on some biochemical techniques relevant to the project; development of a working relationship with other members of the biochemistry section and with Dr. Sayer and his staff at the KETRI primate unit. A second planning trip was conducted during March 1988 and a third working trip is scheduled for July/August 1988.

Tissue culture laboratory: Laboratory space in the biochemistry department was assigned to the project by the Director, Dr. A. Njogu. This laboratory space has been fitted with basic laboratory glassware, microscope and associated minor equipment. Mr. Waithaka has worked with Ms. Maureen Gray of the KETRI Biochemistry Department in establishing a tissue culture laboratory for animal trypanosomes in conjunction with the

laboratory for human pathogens. The establishment of human trypanosome culture awaits the arrival of equipment as noted above.

Strain sensitivity: In vivo studies were begun with 6 isolates from the KETRI trypanosome bank, to determine the susceptibility of these strains to the ODC inhibitor DFMO. All isolates were revived from frozen stabulates and serially passaged 3 times before trials in mice were begun.

These preliminary studies were done to investigate the range of susceptibility to the drug. All isolates were of recent human origin from Uganda (Tororo, Lumino, Busoga) and Kenya (Lambwe Valley, Alupe).

Mice (20-22g) were inoculated with 2×10^5 parasites and treatment was begun 24 hours post infection. DFMO was administered in the drinking water (2% or 4% solutions) for various time periods (3, 6 and 9 days), and animals surviving >30 days beyond the death of the controls were considered cured. A second ODC inhibitor 3,4-dehydromonofluoromethylornithine methyl ester (MFMO.CH₃) was also used in these studies. Results with the KETRI isolates were compared to a standard isolate (T. b. brucei EATRO 110) known to be susceptible to both agents. A wide spectrum of strain-susceptibility to these agents has emerged from these studies.

a) KETRI 2562 and 2772 strains of T. b. rhodesiense appear to be more sensitive both to DFMO and to MFMO.CH₃ in that 100% cure rates were obtained with 2% DFMO for 6 days and 60-100% cure rates were obtained with 0.5% MFMO.CH₃ for 6 days.

b) KETRI 2537, the parasite used in the primate CNS model developed by Dr. P. Sayer, was completely refractory to DFMO at 2% for 3 days and MFMO.CH₃ at 0.5% for 3 days. KETRI 2545 was also highly refractory to both compounds.

c) KETRI 2285 proved to be unique in that although it was highly refractory to 2% DFMO for 6 days, it was susceptible to 0.5% MFMO.CH₃ for 3 days.

d) These studies support the concept that among various T. b. rhodesiense isolates there is a wide range of susceptibility to ODC inhibitors. These findings parallel clinical results which showed that there is a highly variable response to DFMO among cases of T. b. rhodesiense.

Combination therapy: One of the basic scientific concepts of the proposals is the value of using existing drugs in combination with DFMO to reduce dose levels and duration of treatment and

thereby both increase efficacy and reduce toxicity. Such synergism has been demonstrated in animal studies as described in the proposal. Preliminary studies indicated that treatment of KETRI 2562 with DFMO (0.25% for 3 days) and Mel-B (0.5 mg/kg iv/day for 3 days) led to a 60% cure rate. Neither drug used singly at this dose was effective.

Measurement of ODC activity in trypanosomes: A major goal of the investigation is to determine the nature of DFMO refractivity in T. l. rhodesiense strains. To develop this objective we have begun kinetic studies of T. b. rhodesiense ODC with respect to inhibition by DFMO. These studies, begun with Wellcome CT and EATRO 105 strains, demonstrated that the K_i values for DFMO were similar for the susceptible T. b. brucei EATRO 110 (52 μ M) and the T. b. rhodesiense (26 and 28 μ M).

Since impaired uptake of an antimetabolite is very often the basis of resistance, we have begun measurement of DFMO uptake by bloodstream trypomastigotes in vitro using [3 H]DFMO. Both the Wellcome CT and EATRO 105 strains accumulated the drug at the same rate as the DFMO-sensitive T. b. brucei (3100 to 3300 counts/min/mg protein/hr). These studies are preliminary, yet serve to illustrate the complexity of the nature of resistance in T. b. rhodesiense and the need for emphasis on drug combination studies.

Pharmacokinetic studies in primates: During the initial visit of the US investigators (February/March 1987) a preliminary dosing study was conducted using 2 vervet monkeys from the KETRI colony. DFMO was administered at 100 mg/kg q.i.d. (equivalent to the dose used in humans) for 3 days. Ten minutes before the final dose, the animals blood and CSF were collected. The final dose was given by gavage and blood and CSF samples were taken at the times indicated in the table. The samples were analyzed in New York since the required HPLC equipment is not available at KETRI. The techniques used for analysis will be published as a joint US/KETRI paper. The data are shown in the attached table. The peak DFMO levels detected were 160-200 μ M in plasma and 6-8 μ M in CSF. Two additional monkey experiments involving 9 animals have been completed and await DFMO analysis in New York. The results of these pharmacokinetic studies will guide efforts to produce minimal therapeutic drug levels in serum and brain as determined from tissue culture studies to be done at KETRI. This will allow dosing to produce minimum toxicity with DFMO alone and as well as prediction of the required DFMO dose when it is used with other drugs. Additional studies will be done in infected

monkeys to determine if infection alters the drug distribution particularly in the brain since infection produces encephalitis.

Immediate Plans:

A. **Tissue Culture Laboratory:** Equipment to fully equip the tissue culture laboratory is on order and is expected to be delivered and set up by the end of April. Mr. Waithaka will put 6 strains of T. b. rhodesiense in tissue culture and minimum effective doses of DFMO, Mel-B and suramin will be determined during July and August 1988. The US investigators will be at KETRI for 5 weeks during July and August to assist in this and to receive training in tissue culture of African trypanosomes.

B. **In vivo Drug Studies:** Both non-CNS and CNS rodent models will be used to test the accuracy of the predicted drug sensitivities of T. b. rhodesiense strains made with the tissue studies.

C. **Primate Pharmacokinetic Studies:** These will continue and doses will be refined. Comparisons will be made in the pharmacokinetics of infected and non-infected animals to determine if trypanosome-induced encephalitis causes any change in drug distribution.

D. **Primate Drug Trials:** Using the data collected from the tissue culture studies, the mouse studies and the monkey pharmacokinetic studies, predictions will be made for the minimum curative drug doses in monkeys. These predictions will be tested.

Financial Report:

A request for the initial cash advance is attached on form SF 1034. This request includes funds to reimburse KETRI for monies expended to initiate the project and for expenses that can be anticipated through 30 September 1988.

List of Expended Funds:

1. Airfares for two US scientists to travel to work in Kenya in 1987: \$4383
2. Per diem for 2 US scientists working in Kenya 17 February - 19 March (31 days @ \$28.00): \$1736
3. Excess baggage for return trip for US scientists 1987: \$300
4. Airfares for two US scientists to work in Kenya in 1988: \$6482.
5. Per diem for 2 US scientists working in Kenya 9 - 26 March (18 days @ \$28.00): \$1008
6. Simon Gould salary for 1987 (50% effort): \$6000
7. Henry Waithaka salary for 1987 (100% effort): \$7500
8. Equipment and supply order to Fisher Scientific (see attached quote for individual items): \$20,034
9. Miscellaneous supplies purchased in Kenya in 1987: \$701
10. Automobile rental March 11 - 25: \$550
11. Administrative support from KETRI October 1, 1986 - March 31, 1988: \$3000

Total of Expended Funds: \$51,694

Currently anticipated expenditures through 30 September 1988:

1. Airfares for 2 US scientists for July and August 1988: \$6800
2. Perdiem for 2 US scientists for 45 days in July August 1988
@\$28: \$2520
3. Laboratory supplies to be purchased in the next 6 months:
\$5000
4. Equipment to be purchased immediately:
 - a. Nikon inverted phase contrast microscope: \$2000
 - b. Vehicle (Peugeot 505 estate): 12,000
5. Salaries \$7300
6. Administrative support from KETRI for 6 months: \$1000

Total Anticipated Costs: \$36,620

**α -DFMO Levels Measured in Serum and CNS from Monkey Trials
Project USAID**

| No. | Time | Animal No. 35 | | Animal No. 50 | |
|-----|---------|------------------------------------|----------------------------------|------------------------------------|----------------------------------|
| | | Serum α -DFMO (μ M) | CNF α -DFMO (μ M) | Serum α -DFMO (μ M) | CNF α -DFMO (μ M) |
| 1 | -10 min | 54.6 | 5.2 | 60.25 | 4.0 |
| 2 | 15 " | 44.7 | | 73.7 | |
| 3 | 30 " | 57.4 | | 54.5 | |
| 4 | 45 " | 53.5 | | 70. | |
| 5 | 60 " | 65.6 | 7.8 | 94.5 | 6.3 |
| 6 | 3 h | 271.6 | 4.5 | 164. | 5.5 |
| 7 | 6 " | 149. | 2.1 | 116. | 3.7 |
| 8 | 12 " | 100. | 4.0 | 77.4 | 3.9 |
| 9 | 24 " | 45. | 3.7 | 45.6 | 2.7 |
| 10 | 72 " | 24.6 | 2.1 | 25.8 | 1.1 |
| 11 | 7 days | 12. | ND | 27.1 | ND |
