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REPORT NUMBER III

Chemotherapeutic Studies on Schistosomiasis (U)

Annual Technical Report
July 1975 - June 1976

Dr. Aluizio Rosa Prata, M. D.
LTC Myron G. Radke, MSC
July 1976

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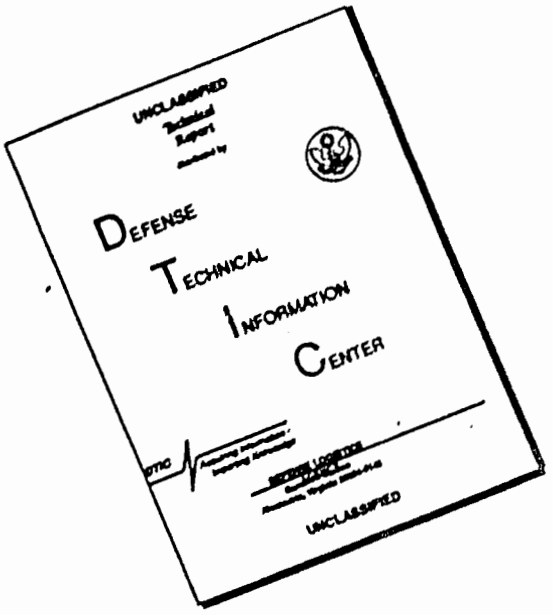

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SUMMARY

The University of Brasilia and The U. S. Army Medical Research Unit (WRAIR) Brasilia have undertaken jointly to find new prophylactic and therapuetic drugs that can be used to minimize the health risk of schistosomiasis.

The WRAIR U. S. Army's Anti-schistosomal Drug Development Program submits selected compounds for prophylactic and therapeutic testing against schistosomiasis mansoni in mice. Drugs are tested initially for prophylactic activity by the mortality test which uses mice that are exposed to a lethal dose of 3,000 or more S. mansoni cercariae. Drugs are prepared in peanut oil at 1280 mgs/kg and are administered subcutaneously in a single inoculation two days after the cercarial exposure. Some selective and all active compounds are further screened by the primary curative test which uses mice exposed to 200 cercariae, and 30-35 days later, drugs are administered orally in five equal daily dosages. Prophylactic drug activity is measured by mouse survival and curative drug activity is measured by increased numbers of live/dead worms in the liver.

The anti-schistosome drug test system calls for maintenance of a large laboratory Biomphalaria glabrata snail colony. Thus, the snail colony's productivity configuration is geared towrads keeping a daily average number of 1,000 S. mansoni cercarial shedding snails. The weekly two million cercarial collections are used to infect the experimental animals for each test group of 50 compounds.

During FY 76, 1,478 selected WRAIR bottle number drugs were tested for anti-schistosomal prophylactic activity The test results were as follows: 1) 1091 drugs were negative, 2) 377 drugs were toxic, and 3) 10 drugs were active (all actives were discreet drugs). All sixteen drugs screened in the primary curative test were found inactive.

The UnB/USAMRU-Brasilia anti-schistosome drug testing facility will continue to screen related active and special synthesized drugs for prophylactic and therapeutic activity as provided by the WRAIR Anti-Schistosomal Drug Development Program (WRAIR).

FOREWORD

The research program of the University of Brasilia and the U. S. Army Medical Research Unit (WRAIR)/Brasilia is to test drugs for prophylactic activity against schistosomiasis mansoni.

The research project is carried on under the following project and task number:

3A762759A831, Task 00, Work Unit 086

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", DHEW Publication Number (NIH) 73-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

The findings in this report are not to be construed as an official Department of the Army Position unless so designated by other authorized documents.

ALUIZIO ROSA PRATA, M.D.
Professor of Tropical Medicine

BODY OF REPORT

PROJECT NO. DAOB 7294
3A762759A831: Tropical Medicine

TASK NO. 00: Tropical Medicine

WORK UNIT 086: Chemotherapeutic Studies on Schistosomiasis

DESCRIPTION:

Drug testing for the schistosomiasis drug development program is performed at the U. S. Army Medical Research Unit/Brasilia. Selected and synthesized compounds are submitted by the Division of Medicinal Chemistry, WRAIR, to our drug testing facility for evaluation. Fifty drugs are tested weekly for prophylactic activity against schistosomiasis mansoni by the mouse mortality test system (Radke, et. al., 1971*). Our routine prophylactic drug testing program was augmented in May 76 to include a curative test. Presently, there are no drugs available to prevent schistosomiasis and the few therapeutic drugs being used are expensive and toxic. Thus, any new prophylactic and therapeutic drugs found or developed by the U. S. Army's anti-schistosomal drug development program would minimize the risk of disease in the event that it would be necessary to deploy U. S. Military and DOD Civilians in endemic areas. Drug advances in the treatment of schistosomiasis would result in reducing government expenditures of men and monies for schistosomiasis control which is endemic to the following geographic areas: South America, Caribbean, Africa, Middle East and Far East. The expertise of the U. S. Army research in schistosomiasis drug development program is directed towards finding drugs which will prevent and cure the disease. The USAMRU/Brasilia test facility (having animals, snail and disease life cycle, and various test system capabilities) in support of the WRAIR, Medicinal Chemistry (source of drugs, synthesized drugs, and automatic data processing) affords unique opportunities for developing and/or finding new drugs to control schistosomiasis.

*Radke, M. G., Broome, P. B., and Belanger, G. S.,: Schistosoma mansoni: Mouse Mortality Test System for Mass Screening for Prophylactic Drugs. Exp. Parasit. 30: 1-10, 1971.

PROGRESS:

a. Laboratory Facility. The final equipment installation was completed with the 80 gallon air compressor which delivers oil/water free air to the Biomphalaria glabrata snail colony. The delay in installing the special air compressor was a result of the 220 volt, three phase electric requirement. A special transformer was purchased to change the line voltage of 380 volts, three phase to 220 volts, three phase.

b. Animal Facility. Four air conditioners were installed in the Bioterio's mouse breeding facility to provide a uniform day/night temperature. By establishing a uniform ambient temperature, it is hoped that the mouse colony production will be stabilized. Our drug testing unit receives from the University of Brasilia's Bioterio 400, 18-23 gram mice weekly. Beginning FY 77, the mouse deliveries requested are 500/600 weekly.

c. Snail Colony. The weekly laboratory Biomphalaria glabrata snail colony production is 500, 5 - 8 mm snails of which 350/400 are individually exposed to 8 - 12 S. mansoni miracidia. Eighty-two percent of the miracidia exposed snails are surviving at 42 days (monthly snail survival was 67 to 88 percent) with a snail infection rate of 56 percent (monthly snail infection rate was 40 to 77 percent). The daily number of S. mansoni cercariae shedding snails maintained was 1,239 (the daily average number of infected snails maintained per 30 days was 992 to 1,499). These infected snails provided weekly cercarial collections of 3 to 5 million. Our weekly cercarial usage is two million.

d. Drug Testing. Thirty drugs were tested weekly at two dosages, 640 and 1920 mgs/kg. However, many compounds were found toxic at 1920 mugs/kg and the limited 500 milligram samples forced us to lower the test dosage. In December 1975, the drug test facility began testing fifty compounds weekly at 1280 mgs/kg.

TEST PROCEDURE:

All experimental drugs are screened for prophylactic activity by the mouse mortality test system. Mice weighing 18-23 grams (39-43 days old) are exposed 45 minutes by tail immersion to 3,000 or more S. mansoni cercariae. Drugs are formulated in peanut oil at either 640 and 1920 mgs/kg or 1280 mgs/kg. Five mice are used per test

dosage and drugs are administered subcutaneously two days after cercarial exposure. Any toxic drugs, that is infected treated mice dying within the first 10 days of infection, are retested at 40 and 160 mgs/kg. The number of compounds tested weekly was increased from 30 to 50 when a lower maximum drug dosage of 1280 mgs/kg was begun. A routine test group for 50 selected compounds consisted of 315 mice; ten mice are normal, and 305 mice are exposed to 3,000 or more cercariae. The two hundred fifty (250) infected mice are used to screen 50 candidate drugs with five mice per drug, five mice are given Niridazole (reference drug), and fifty mice are untreated infected controls. An active drug is one in which treated mice survive 49 days after cercarial exposure. The infected control mice start dying on the 20th day and by the 30th day all are dead. Drug activity is based upon the number of surviving mice and worm burden data as obtained by perfusing the living treated mice on the 49th day of infection (Radke, et al., 1961**). In May 1976, a curative test system was added to the anti-schistosome drug testing program. Some selected drugs and all prophylactic active drugs are tested for therapeutic activity against mice exposed to 200 S. mansoni cercariae. Treatment of the infected mice begins, when adult worms are present, 30 to 35 days after cercarial exposure by administering the drug orally in five equal daily dosages. Drugs are prepared in peanut oil for oral administration at either 50, 100, 160 or 320 mgs/kg. Five mice are used for each drug level. Mice are sacrificed 10 days after completing drug treatment. The liver is removed, squashed between two glass plates, and the number of live and dead worms are counted. Worms are considered dead when the addition of Serotonin fails to induce motility. A curative test group consists of several test drugs (five mice per drug), 15 infected non-treated control mice, and ten reference drug (Oxanmiquine) treated mice. Drug activity is demonstrated by an increase in the number of live worms in the liver ("liver shift") and the presences of dead worms in the liver.

RESULTS:

During FY 76, a total of 1,290 selected WRAIR bottle numbered drugs were tested at dosages: 1) 640 and 1920

**Radke, M.G., Berrios-Duran, L.A. and Moran, K.,: A perfusion Procedure (Perf-O-Suction) for recovery of Schistosome Worms. J. Parasit. 47: 366-68, 1961.

mgs/kg, or 2) 1280 mgs/kg against schistosomiasis mansoni by the mouse mortality test. An additional 188 drugs were retested at other dosages because of either drug toxicity or drug activity. The test results were: 1) 1091 negative drugs, 2) 377 toxic drugs, and 3) 10 active drugs (See Figure 1). A total of 16 drugs were tested in the curative test procedure. The liver squashes from four drugs had more than 10 live worms per mouse (infected control untreated mice have around five worms). However, dead worms found in the liver squash preparation from drug treated mice indicates therapeutic activity.

FIGURE 1

List of Active Drugs against Schistosomiasis *mansoni*

by the Mouse Mortality Test System

	WRAIR
No.	Drug Bottle No.
1.	BG 21 253
2.	BE 43 811
3.	BE 18 747
4.	BE 18 774
5.	BG 22 303
6.	BG 22 223
7.	AY 98 670
8.	BG 43 731
9.	BG 44 023
10.	BG 47 471

The above bottle numbered drugs are commercially discreet compounds and the disclosure of the structure is prohibited by law until furnishing company's obtain U. S. Government Patents.

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The University of Brasilia and USAMRU (WRAIR) Brasilia tested 1,478 compounds for prophylactic and curative activity against schistosomiasis in mice. The drugs were screened for prophylactic activity by the mortality test system. The test results were: 1) 1091 drugs were negative, 2) 377 drugs were toxic, and 3) 10 drugs were active (all discreet compounds). The sixteen drugs tested in the primary curative test were inactive.

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