



REPORT NUMBER THREE

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CHEMOTHERAPY OF TRYPANOSOMA RHODESIENSE

FINAL REPORT

DORA S. RANE

For the period of June 1, 1974 to September 30, 1975

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Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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CONTINUATION OF THE SCREENING PROCEDURE FOR THE EVALUATION OF TRYPANOSOMICIDAL ACTIVITY OF CANDIDATE COMPOUNDS USING TRYPANOSOMA RHODESIENSE INFECTIONS IN MICE

The test system described herein was developed specifically to evaluate the trypanosomicidal activity of large numbers of candidate compounds.* Based on blood-induced <u>Trypanosoma</u> <u>rhodesiense</u> infections in mice, it performs as a primary screen or as a secondary screen and confirmatory test and gives precise quantitative evaluations of chemical compounds that demonstrate potentially useful therapeutic and/or prophylactic activity in <u>T</u>. <u>rhodesiense</u> infections. Consequently, it is also a helpful guideline in the synthesis of new active agents.

These agents include: (1) chemicals structurally related to compounds of known value in the treatment or prevention of <u>T</u>. <u>rhodesiense</u> infections; (2) chemicals structurally unrelated to compounds of known value in the treatment or prevention of <u>T</u>. <u>rhodesiense</u> infections and; (3) structural analogues of compounds that have demonstrated activity in our screening procedure and represent novel chemical groups.

All candidate compounds were obtained from the Department of Medicinal Chemistry at the Walter Reed Institute of Research.

Table I summarizes the number of compounds tested and the number of mice used from August 1, 1972, through September 30, 1975.

Our own colony of ICR/HA Swiss mice provided all the test animals needed in this operation. Using mice of a given age, sex and weight and a standard inoculum of the Wellcome CT-strain of <u>T</u>. <u>rhodesiense</u>, it has been possible to produce a consistently uniform disease fatal to 100 percent of untreated animals within 4-6 days.

Test compounds were administered either parenterally or orally in a single dose on the day of infection.

Activity was determined by responses to candidate compounds by <u>T. rhodesiense</u> infections in mice as expressed in comparisons of the maximum survival time of the treated trypanosome-infected animals and the survival time of the untreated trypanosome-infected controls. To be classified as active, a compound must suppress the disease and produce an increase of at least 100 percent in the life span of the treated animals over that of the untreated controls.

* Designed, developed and operated by Dr. Leo Rane until 1973, then operated by Mrs. Dora Rane until 1976.

TABLE I

COMPOUNDS TESTED AND MICE UTILIZED AUGUST 1, 1972 - SEPTEMBER 30, 1975

YEAR	NUMBER OF COMPOUNDS	NUMBER OF MICE
AUGUST 1, 1972 - MAY 31, 1973	3,030	51,405
JUNE 1, 1973 - MAY 31, 1974	1,581	25,360
JUNE 1, 1974 - MAY 31, 1975	1,826	33,850
JUNE 1, 1975 - SEPT. 30, 1975	593	11,260
TOTAL	7,030	121,875

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Acceptance of a test compound's activity was also predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED). A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die and a minimum effective dose as the minimum dose increasing the life span of treated animals 100% over the life span of untreated controls.

METHODS

Animal Hosts. Our own breeding colony of ICR/HA Swiss mice has supplied all the animals used in this screening procedure. Test animals weigh 30-32 grams, weight variations in any given experimental or control group being carefully limited to 3 grams. In all tests animals have been of the male sex and approximately of the same age.

Animals on test are housed in metal topped plastic cages, fed a standard laboratory diet and given water ad lib.

Once the mice have been given a drug they are kept in a room maintained at 84° F (+ 2° F) and a relative humidity of 66% (+ 2%).

Test Procedure. Test animals receive an intraperitoneal injection of 0.5 cc of a 1:50,000 dilution of heparinized heart blood drawn from a donor mouse infected 3 days earlier.

The donor line is maintained by 3 day blood passes, each animal receiving 0.1 cc of a 1:500 dilution of heparinized heart blood drawn from a 3 day donor. Donors, like test animals, weigh 30-32 grams, weight variations for each pass being limited to 3 grams.

To check factors such as changes in the infectivity of our <u>T</u>. <u>rhodesiense</u> strain, or in the susceptibility of the host one group of infected untreated mice are included as negative controls. In order to determine the effect a drug exerts on a trypanosome infection two parameters are measured; the first being an increase in survival time, and the second concerns curative action. For comparative purposes standard compounds, stilbamidine isethionate and 2hydroxystilbamidine isethionate are administered at one level each (26.5 mgs/kg) to separate groups of 10 mice which serve as positive controls producing definite increases in survival time and curative effects. Another function of these two positive controls involves a check on whether three procedures are performed correctly; the drug weighing, the preparation of drug solutions or suspensions and the administration of drugs. Drug Administration. Test compounds are dissolved or suspended in peanut oil and prepared in three or more graded doses. At least three different doses of each test compound are included in an experiment. Groups of 5 mice per dose level of drug are utilized.

Treatment consists of a single dose administered subcutaneously or orally on the day of infection. Deaths that occur before the fourth day, when untreated controls begin to die, are regarded as the result of a compound's toxic effect and not as the result of action by the infecting parasite.

Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice. However, if an active drug is toxic for the host, the toxicity of this compound may become a limiting factor to changes in dose levels.

Treated animals alive at the end of 30 days are considered as cured.

<u>Drug Activity</u>. An increase of 100% in survival time is considered the minimum significantly effective response for a candidate compound. Clearly inactive compounds are rejected after one test, borderline compounds after two tests.

Active compounds are subjected to a test to determine a dose response curve (6 or 9 different doses) so that the spread between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED) may be established.

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST TRYPANOSOMA RHODESIENSE INFECTIONS IN MICE

During the opening period of this project, June 1, 1972 -May 31, 1973, our screening procedure was developed and its reliability established. 3,030 selected compounds were screened including a number of agents known to be effective in \underline{T} . rhodesiense infected mice.

1,581 compounds were tested in the period June 1, 1973 -May 31, 1974. Of these, 185 demonstrated a degree of activity sufficient to produce at lease 100 percent increases in the survival time of treated <u>T</u>. <u>rhodesiense</u> infected mice, 92 were active subcutaneously and 93 orally. 1,826 compounds were tested in the period June 1, 1974 -May 31, 1975. Of the 298 recognized as active compounds, 225 were active subcutaneously and 73 orally.

593 compounds were tested in the period June 1, 1975 -September 30, 1975. Of the 123 recognized as active compounds, 109 were active subcutaneously and 14 orally.

This breakdown is significant since: (1) activity evaluations provided in our screening procedure are precise and quantitative; (2) dose response curves of active compounds administered subcutaneously show a wider spread between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED) than dose response curves of active compounds administered orally and; (3) these dose responses also reveal a wider spread of toxic effects when active compounds toxic for the host are administered subcutaneously rather than orally.

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