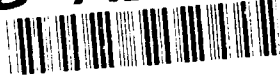


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**DEVELOPMENT OF A NEW CHEMOTHERAPY FOR HUMAN
AFRICAN TRYPANOSOMIASIS BY USING AN ANIMAL MODEL:
SURAMIN WITH DL- α -DIFLUOROMETHYLORNITHINE**

(Chemotherapy for African trypanosomiasis by polyamine biosynthesis inhibition.)

ANNUAL PROGRESS REPORT -- YEAR TWO

Allen B. Clarkson, Jr., Ph.D.

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SUMMARY

OVERVIEW OF PROJECT: The general objective of this contract was to provide a preclinical evaluation of the potential of eflornithine (DL- α -difluoromethylornithine, DFMO, Ornidyl[®]) in combination with suramin as a treatment for human *Trypanosoma brucei rhodesiense* infections (Rhodesian African Sleeping Sickness). Since this disease progresses rapidly, a useful therapy must be active against both the early and late stages of the disease; i.e., both prior to and after the central nervous system (CNS) becomes infected. The tools used to evaluate efficacy of the therapy were mouse models of both early and late stage *T. brucei rhodesiense* Sleeping Sickness. The project also included a study designed to detect potential adverse interactions between these two drugs. The combination was effective against *T. brucei rhodesiense* that was resistant to these drugs when given individually. There were no adverse interactions detected.

PROGRESS: *Objective 1:* Establishment of a central nervous system (CNS) *T. brucei rhodesiense* infection model to be used for drug testing. This had been accomplished in year 1. *Objective 2:* Establishment of the maximum tolerated dose of DFMO. This had been accomplished in year 1. *Objective 3:* Excluded from the final contract per USAMRDC. *Objective 4:* Evaluation of eflornithine and suramin individually in the mouse CNS model. The mouse CNS model did not respond to either drug alone. *Objective 5:* Evaluation of a combination of eflornithine and suramin in the CNS model. The combination was effective thus confirming the basic hypothesis of the proposal. *Objective 6:* Evaluation of a combination of eflornithine and suramin in an early stage model. The combination was effective as predicted. *Objective 7:* Examination of multiple stocks of *T. brucei rhodesiense*. The contract was modified per USAMRDC so that this objective was not given high priority. The objective was achieved partially through collaboration with another laboratory. The results indicate a wide range of drug sensitivity in "wild" isolates from human cases of Rhodesian Sleeping Sickness. This finding will be of importance in any future clinical trials for this disease. *Objective 8:* Excluded from the final contract per USAMRDC. *Objective 9:* Study of possible adverse interactions between eflornithine and suramin. This was completed and none were found. *Objective 10:* Excluded from the final contract per USAMRDC.

FOREWORD

Citations of commercial organizations or trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

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INTRODUCTION

Present capabilities to treat cases of African Sleeping Sickness are inadequate. This is especially true of those cases caused by *Trypanosoma brucei rhodesiense*. Therefore more efficacious and less toxic drugs are needed. This study is a preclinical evaluation of an inhibitor of a crucial enzyme of polyamine biosynthesis in combination with an old drug used for Sleeping Sickness. The ultimate objective is to develop a new and effective treatment based on interference with polyamine function of these parasites.

Studies with the exquisitely specific polyamine biosynthesis inhibitor eflornithine (DL-a-difluoromethylornithine, DFMO, Ornidyl[®]) showed that this drug cures mice with acute *T. brucei brucei* (strain TREU 667) infections; i.e., the stage of infection before the central nervous system has become involved (Bacchi, et al., 1980; McCann et al., 1984). Eflornithine does not produce a 100% cure rate in mice with late or chronic *T. b. brucei*-infections; i.e. after the parasites have invaded the CNS (Clarkson, et al., 1983; Clarkson, et al., 1984). However, initial (Sjoerdsma and Schechter, 1984) and subsequent extensive clinical trials (Schechter et al., 1987) have shown it to cure patients infected with *T. brucei gambiense* (West African or Gambian Sleeping Sickness) in both early and late cases. The limited number *T. brucei rhodesiense* (East African or Rhodesian Sleeping Sickness) cases treated with eflornithine has indicated that it is not as effective for *T. brucei rhodesiense* as it is for *T. brucei gambiense*. According to Dr. Peter de Raadt, recently responsible for the trypanosomiasis section of the World Health Organization TDR program, about a dozen *T. brucei rhodesiense* patients were treated with eflornithine in Zimbabwe and most were not cured. In Kenyan trials against *T. brucei rhodesiense* using twice the dose of eflornithine as used for *T. brucei gambiense* infections, only 3 of 7 *T. brucei rhodesiense* patients treated with eflornithine have been cured. There is one report on eflornithine treatment of a mouse model of *T. brucei rhodesiense* and this report indicated that that strain of *T. brucei rhodesiense* responds to eflornithine (McCann et al., 1981).

Previous work has shown that eflornithine acts synergistically with other anti-trypanosome drugs (Bacchi et al., 1982; Clarkson et al., 1983; Clarkson et al., 1984). For example, the inability of eflornithine to cure late stage *T. brucei brucei* (TREU 667) infections in mice can be overcome by using eflornithine in combination with either the anticancer drug bleomycin (Clarkson et al., 1983) or with suramin, an established trypanocidal drug (Clarkson et al., 1984). These combinations provide a 100% cure rate in mice with late stage *T. brucei brucei* (TREU 667) disease although neither bleomycin nor suramin alone, like eflornithine, will cure these animals. The combination of drugs also allows the dose of each drug to be greatly reduced compared to the dose when used singly for non-CNS infections.

The enhanced action of a combination of eflornithine with another trypanocidal drug offers a real possibility of extending eflornithine therapy to patients infected with *T. brucei rhodesiense*. A combination of eflornithine, which is well tolerated by patients at the doses given for *T. brucei gambiense*, with suramin is more promising than one with bleomycin. Bleomycin is a toxic and expensive drug that would not be easily used under the conditions Sleeping Sickness treatment is given. Suramin, however, is generally well tolerated despite sporadic incidences of toxicity. The dose of suramin required for successful combination with eflornithine is only 4% of the total suramin dose (mg/kg) used for human non- CNS disease. Therefore the combination of suramin and eflornithine may well provide a safe and effective treatment for all stages of *T. brucei rhodesiense* infection.

The work reported here is progress made in a feasibility study undertaken to investigate the potential of the combination of suramin and eflornithine for treatment of early and late stage *T. brucei rhodesiense*. This is a preclinical study utilizing mouse models of early and late stage disease. Since the response of *T. brucei rhodesiense*-infected patients has not been consistent, this study uses multiple stocks of *T. brucei rhodesiense* isolated from patients in East Africa to determine the spectrum of sensitivity to the drugs singly and in combination. The study includes preliminary experiments designed to detect any obvious adverse interaction between the two drugs.

RESULTS

Objective 4: Evaluation of eflornithine and suramin individually in the mouse KETRI 2537 CNS model. The CNS model was highly resistant to these drugs used individually. The data are presented in Table 1.

Objective 5: Evaluation of a combination of eflornithine and suramin in the CNS model. The combination was 100% effective thus confirming the basic underlying hypothesis of the proposal. The data are presented in Table 2.

Objective 6: Evaluation of a combination of eflornithine and suramin in an early stage model. The combination was also effective in an early stage model at very low suramin doses. The data are presented in Table 3.

Objective 7: Examination of multiple stocks of *T. brucei rhodesiense*. This objective was not given high priority in the contract. It was achieved partially through efforts in the contracting laboratory and partially through collaboration with a laboratory headed by Cyrus Bacchi. The results indicate a wide range of drug sensitivity in "wild" isolates from human cases of Rhodesian Sleeping Sickness and this is a finding that should be considered in the design and interpretation of clinical trials.

Some of the data gathered using an acute model of Rhodesian Sleeping Sickness are presented in an attached reprint "Differential Susceptibility to DL- α -difluoromethylornithine in clinical isolates of *Trypanosoma brucei rhodesiense*" Bacchi, C.J., Nathan, H.C., Livingston, T., Valladares, G., Saric, M., Sayer, P.D., Njogu, A.R. and Clarkson, A.B., Jr., *Ant. Microbial. Agents. Chem.*34:1183-1188 (1990).

The seven stocks of *T. b.rucei rhodesiense* examined for suitability for mouse models were also examined for sensitivity to eflornithine. Separately collected data from six of these seven are included in Bacchi *et al.*, *op. cit.*. Groups of 5 mice were inoculated with 25,000 parasites and the groups were treated 24 hours after inoculation. Treatment was with 2% or 4% eflornithine in the drinking water for 3, 6 or 9 days. The data for the acute models (Table 5) show that this series of parasite stocks exhibits a range of sensitivities to eflornithine as shown by Bacchi *et al.*, *op. cit.*. Treatment with 2% for 6 days provided a much higher cure rate than treatment with 4% for 3 days although the total amount of eflornithine administered was similar. This emphasizes the importance of the duration of treatment over total dose. Although the sensitivity of the various *T. brucei rhodesiense* strains to eflornithine differed, if adequate dosage were given all seven of these strains could be treated successfully with eflornithine in the acute stage in agreement with the data in Bacchi *et al.*, *op. cit.*

Objective 9: Study of possible adverse interactions between eflornithine and suramin. Two series of mice were administered suramin in a graded dose schedule. Each series contained nine groups of ten randomly assigned mice per group. The groups received daily suramin injections for 0, 1, 2, 3, 4, 5, 6, 7 or 8 days. Each suramin dose was 40 mg/kg i.v. to yield total doses of 0, 40, 80, 120, 160, 200, 240, 280 or 320 mg/kg. One series was administered 2% eflornithine in the drinking water beginning with the initiation of the suramin treatment and continuing until the time of sacrifice. The other series was given no eflornithine. The animals were sacrificed 14 days after the last suramin dose. In the series given eflornithine, a group of mice was given no suramin and was treated with 2% eflornithine for 14 days and then sacrificed.

The objective of the experiment was to determine if the minimal toxic dose of suramin was lower when 2% eflornithine was administered simultaneously. While there is very little data on suramin toxicity in the literature, the doses of suramin chosen were based on a published LD₅₀ of 40 mg/kg i.v. (Merck Index, the only source found). The maximum dose used was 320 mg/kg or eight times the published LD₅₀. There were no apparent toxic effects when suramin was combined with eflornithine despite the very high dosage of suramin. In the entire experiment there was one spontaneous death. This occurred in the group given 2% eflornithine and a total suramin dose of 200 mg/kg. Since this is in the middle of the suramin dose range and the death occurred 9 days after the last suramin dose, this death cannot be attributed to suramin/eflornithine toxicity. There were no signs of diarrhea in any of the treated animals nor any fur ruffling which is generally a first sign of toxicity or sickness in mice. There was no difference among any of the groups in hematocrits taken at the time of sacrifice. Similarly, there was no difference in mouse weight gain during the treatment period. A pooled 24 hour urine sample was collected from each group just before sacrifice and no group showed any proteinuria which would be expected if eflornithine enhanced suramin nephrotoxicity, the most common toxic effect of suramin. A full pathological study was not made but mice given the highest doses of drugs showed no clear change in gut endothelium. Although small animals often both require and tolerate higher drug doses than larger animals, this is usually due to the faster drug clearance rate of small animals. Since suramin binds to albumin and is cleared only slowly in both small and large animals, dose responses to suramin, both therapeutic and adverse, are not likely to be extremely different.

Since we observed no suramin toxicity even at the highest doses used, we cannot rule out the possibility that eflornithine could have exacerbated such toxicity had it occurred. However, we can be confident that there is no obvious adverse interaction between these drugs at the very high doses tested. This is a very encouraging finding and supports the eventual development of this combination as a clinical protocol.

DISCUSSION

The efficacy data collected under this contract support the hypothesis that a combination of suramin and eflornithine will be an effective therapy for human *Trypanosoma brucei rhodesiense* infections.

These data are also encouraging in that eflornithine in combination with suramin was well tolerated even at very high doses. Despite the administration of suramin at eight times the expected LD₅₀, no toxicity was observed either alone or in combination with eflornithine. Suramin toxicity is, however, a problematic parameter to measure and the mouse may not be an ideal model. There is no animal model for suramin toxicity nor is the basis of this toxicity understood. Therefore, it is likely that the question of possible exacerbation of suramin toxicity will only be answered by careful collection of clinical data on the frequency of adverse suramin reactions when used alone and when used in combination with eflornithine. Clinical trials of eflornithine with suramin are proceeding in Tanzania under WHO guidance in cooperation with Marion, Merrell-Dow (Peter de Raadt, WHO) although no data are yet available.

The data collected under this contract also demonstrate the importance of considering the natural variation in the sensitivity of "wild" clinical isolates to various drugs and drug candidates. It is reasonable to conclude that natural human infections present at least as great a range of drug sensitivity as we observed among the isolated studied. It would, therefore, be very misleading to evaluate a drug candidate using a single isolate and even more misleading to use an "old" laboratory strain or a clone.

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TABLE 1

Treatment of CNS infection with *T. brucei rhodesiense* strain KETRI 243 with diminazene acetate, suramin and eflornithine used as single agents

Mice were infected with 1.0 (10^5) parasites 21 days prior to initiation of treatment. Diminazene acetate (Berenil[®]) and suramin were given as single doses at the indicated mg/kg. Eflornithine was given in the drinking water at the indicated concentration beginning on day 21 and continuing for the indicated number of days.

KETRI 243

Diminazene acetate (single dose on day indicated)	Suramin (single dose on day 21)	Eflornithine (continuously in drinking water from day 21)	# Cured of # Treated
40 mg/kg day 7 40 mg/kg day 21			5 of 5 1 of 5
	20 mg/kg		0 of 5
		2% for 14 days 4% for 14 days 6% for 14 days	0 of 5 1 of 5 0 of 5
		2% for 28 days 2% for 28 days 6% for 28 days	0 of 5 2 of 5 2 of 5

TABLE 2

Treatment of CNS infection with *T. brucei rhodesiense* strain KETRI 243
with suramin and eflornithine used in combination

Mice were infected as described for Table 1 and treatment was begun three weeks after inoculation. Surmain was given as a single treatment at the indicated dosage at the beginning of the treatment period. Eflornithine was given in the drinking water at the indicated concentration for the indicated number of days.

KETRI 243

Suramin (single dose on day 21)	Eflornithine (continuously in drinking water from day 21)	# Cured of # Treated
20 mg/kg	2% for 14 days	5 of 5
20 mg/kg	4% for 14 days	5 of 5
20 mg/kg	6% for 14 days	5 of 5
20 mg/kg	2% for 21 days	5 of 5
20 mg/kg	4% for 21 days	5 of 5
20 mg/kg	6% for 21 days	5 of 5

TABLE 3

Treatment of acute infection with *T. brucei rhodesiense* strain KETRI 243

KETRI 243 (A) and 2538 (B) are moderately resistant and highly resistant, respectively, to eflornithine when used as a single agent (Bacchi *et al.*, 1990). Mice were infected with 2.5×10^5 parasites 24 hours prior to initiation of treatment. The mice were examined for relapsing parasitemia for nine weeks post infection; the last relapse occurred three weeks after infection.

A. KETRI 243

Suramin (single dose on day 21)	Eflornithine (continuously in drinking water from day 21)	# Cured of # Treated
1 mg/kg	2% for 2 days	2 of 5
1 mg/kg	2% for 8 days	5 of 5
1 mg/kg	4% for 2 days	4 of 5
1 mg/kg	4% for 8 days	5 of 5
2 mg/kg	2% for 2 days	5 of 5
2 mg/kg	2% for 8 days	5 of 5
2 mg/kg	4% for 2 days	3 of 5
2 mg/kg	4% for 8 days	5 of 5
5 mg/kg	2% for 2 days	5 of 5
5 mg/kg	2% for 8 days	5 of 5
5 mg/kg	4% for 2 days	5 of 5
5 mg/kg	4% for 8 days	5 of 5
10 mg/kg	2% for 2 days	5 of 5
10 mg/kg	2% for 8 days	5 of 5
10 mg/kg	4% for 2 days	5 of 5
10 mg/kg	4% for 8 days	5 of 5

TABLE 3 (cont.)

B. KETRI 2538

Suramin (single dose on day 21)	Eflornithine (continuously in drinking water from day 21)	# Cured of # Treated
1 mg/kg	2% for 2 days	5 of 5
1 mg/kg	2% for 8 days	5 of 5
1 mg/kg	4% for 2 days	5 of 5
1 mg/kg	4% for 8 days	5 of 5
2 mg/kg	2% for 2 days	5 of 5
2 mg/kg	2% for 8 days	5 of 5
2 mg/kg	4% for 2 days	5 of 5
2 mg/kg	4% for 8 days	5 of 5
5 mg/kg	2% for 2 days	5 of 5
5 mg/kg	2% for 8 days	5 of 5
5 mg/kg	4% for 2 days	5 of 5
5 mg/kg	4% for 8 days	5 of 5
10 mg/kg	2% for 2 days	5 of 5
10 mg/kg	2% for 8 days	5 of 5
10 mg/kg	4% for 2 days	5 of 5
10 mg/kg	4% for 8 days	5 of 5

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