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FOREWORD

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*William L. Hanson* Oct. 19, 1994  
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## INTRODUCTION

Protozoan parasites of the genus *Leishmania* are widespread throughout the world where they cause a complex of visceral or cutaneous diseases in human beings as well as some animals including dogs in numerous tropical and sub-tropical countries (1,2,3). Since the leishmaniases commonly exist as zoonoses, these diseases pose a significant potential threat to military personnel as well as military dogs throughout endemic areas. Relatively recent publicity regarding infection of personnel involved in Operation Desert Storm has reemphasized the military significance of the leishmaniases.

Better drugs are needed for the treatment of the leishmaniases since those currently available are often not satisfactorily effective and are potentially toxic to man and animals.

This laboratory has been involved for several years in studies to identify new compounds for antileishmanial activity against both visceral (*Leishmania Leishmania donovani*) and cutaneous (*Leishmania Viannia braziliensis*) leishmaniasis. Although several new compounds have been identified with activity against *L. (V.) braziliensis*, none have shown adequate promise to warrant initiation of clinical trials. However, among the most promising active compounds found against visceral leishmaniasis during these studies is the 8-aminoquinoline, WRO6026 (4). This compound is now undergoing clinical trials in Kenyan visceral leishmaniasis patients. Testing for compounds active against visceral leishmaniasis has continued during this project period in the event that WRO6026 does not perform in the field as expected and testing has continued to identify more active and less toxic compounds for *L. (V.) braziliensis*. Emphasis was placed on the study of *in vivo* activity of plant derivatives which have been noted previously to be active *in vitro* against *Leishmania*.

This report summarizes the results of studies conducted for this contract during the period March 28, 1994 through September 23, 1994.

## MATERIALS AND METHODS

## I. Visceral Test System

A Khartoum strain of *L. (L.) donovani* (WR378) was used and the golden hamster (*Mesocricetus auratus*), 50-70 gm, served as the host animal. Suspensions of amastigotes for infection of experimental hamsters were prepared by grinding heavily infected hamster spleens in sterile saline in a Ten Broeck tissue grinder and diluting the suspensions so that 0.2 ml contained approximately  $10 \times 10^6$  amastigotes. Each experimental hamster was infected via the intracardiac injection of 0.2 ml of the amastigote suspension.

The testing procedure used was that described by Stauber and his associates (5,6,7) as modified by Hanson, et al. (8) with the exception that the time interval between completion of treatment and termination of the experiment was extended (see next paragraph). On Day 3 following infection, hamsters were divided randomly into experimental groups consisting of a minimum of 6 animals per group, initial group weights were obtained, and administration of test compounds was initiated. Each compound was tested at 2 or 3 drug dosage levels dependent on the priority rating and nature of the compound.

The vehicle for the reference and test compounds was 0.6% DMSO. Each test group contained six hamsters. A control group of six hamsters received the 0.6% DMSO vehicle only and the reference compound, Glucantime®, was given at 2 drug dosage levels, 416 and 208 mg sb/kg. All test compounds were administered at 832 total mg/kg. The reference compound, Glucantime®, was administered via the intramuscular route and the test compounds were administered orally twice daily on days 3 through 6. Final group weights were obtained on all experimental hamsters on Day 7 and all hamsters were killed four weeks after completion of treatment, livers removed, weighed, and liver impressions made for enumeration of amastigotes. Subsequently, the total number of parasites per liver was determined as described by Stauber, et al. (5,6,7).

In addition to recording body weight changes as a general indicator of toxicity of the test compounds, experimental hamsters were observed for such clinical signs of toxicity as nervous disorders, roughened hair coat, and sluggish activity. Deaths of the animals was also considered indicative of significant drug toxicity.

After determining the ratio of numbers of amastigotes per host cell nucleus, the weight of the organ, and initial and final weights of the hamsters, the raw data was evaluated with a Gateway 2000 microcomputer using a program which calculates percent weight change, total numbers of parasites, mean numbers of parasites per organ, and percent parasite suppression. The computer program then performs linear and non-linear regression analysis and calculates an  $SD_{50}$  for active compound from each of the analyses (drug dosage resulting in 50% suppression of amastigotes). The  $SD_{50}$  from the non-linear analysis is used for a comparison of the relative efficacy of the test compounds and the efficacy of test compounds relative to that of the reference compound, Glucantime®. The linear regression analysis is included only for comparison with the non-linear analysis.

## II. Cutaneous Test System

*Leishmania (V.) braziliensis* (WR539) was used in these studies. Male golden hamsters, 50-70 gm, served as experimental hosts.

Promastigotes for establishing experimental infections in hamsters were grown in Schneider's *Drosophila* Medium (Hendricks, et al., 9) and quantitated using procedures described previously (Hanson and Roberson, 10). In preparation for infection and weekly during the experiment, the hair was clipped on the dorsal tail head and a commercial depilatory agent applied to the area to remove the remaining hair. Each hamster was inoculated via the intradermal route with approximately  $1.5 \times 10^7$  promastigotes of *L. (V.) braziliensis* near the base of the tail using a 0.25 ml glass syringe equipped with a 30 gauge X 1/2" needle. Each experimental group consisted of six hamsters. Initial body weights were obtained and administration of therapy was initiated on Day 19 postinfection, and continued through Day 22 postinfection. Glucantime® was included at two dosage levels (832 and 208 total mg/Sb/kg) as the reference compound, and a group of six hamsters received vehicle only (0.6% DMSO). Test compounds were administered at 780 total mg/kg. The reference compound, Glucantime®, was administered via the intramuscular route and the test compounds were administered orally.

Lesion area of each experimental hamster was determined one week after completion of treatment and again at eight weeks after completion of treatment with the aid of a template made at WRAIR and calibrated according to the formula  $r_1 r_2 \pi$  where  $r_1$  is the major radius of the lesion and  $r_2$  is the minor radius (Wilson, et al., 11). The mean lesion area of each experimental group was obtained and the percent suppression of lesion size calculated by comparing the mean lesion area of each treated group with that of the group receiving vehicle only with the aid of a computer program and a Gateway 2000 microcomputer. The computer program performs linear and non-linear regression analysis and calculates an  $SD_{50}$  for each active compound using both analyses. The  $SD_{50}$  obtained from the non-linear analyses is used for a rough comparison of the relative efficacies of the test compounds and the relative efficacy of test compounds with that of the reference compound, Glucantime®. The linear regression analysis is performed for comparison with the non-linear analysis.



## RESULTS

Six plant derivatives which have been noted by others to be active *in vitro* against *Leishmania* were noted to be inactive in hamsters when tested against either *L. (L.) donovani* (Table I) or *L. (V.) braziliensis* (Table II). Extension of time up to 4-6 weeks between treatment and evaluation of results did not increase the *in vivo* activity of any of the compounds studied in either test system.

None of the compounds were toxic to hamsters as indicated by mortality, weight loss, or clinical signs.

## DISCUSSION

The purposes of these studies were (1) to determine whether selected plant derivatives with *in vitro* antileishmanial activity were also active *in vivo* in hamsters, and (2) to determine if extending the interval between completion of therapy and evaluation of parasite numbers or lesion size would reveal possible evidence of delayed activity of the test compounds.

Although these compounds had been observed to be active *in vitro*, no evidence of *in vivo* antileishmanial activity was noted in our studies. It is not unusual for test compounds to be active against parasites *in vivo* and not be active *in vitro* which may be due to a variety of factors (12).

Extension of the time interval between administration of the test compounds and evaluation of their effect did not reveal any evidence of delayed activity of the test compounds.

## CONCLUSIONS

1. The probability of identifying compounds with *in vivo* antileishmanial activity is low.
2. Although the compounds selected for these studies were active *in vitro* but not *in vivo*, *in vitro* activity must remain a major criterion for selecting test compounds.
3. Although the plant derivatives selected for these studies were not active, this approach has tremendous merit and should continue since historically considerable success has been achieved with plant derivatives against such parasites as amoebae, *Plasmodium*, and various helminths.

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**Appendix**

**Table I.** Summary of the suppressive activity of selected compounds against *Leishmania Leishmania donovani* four weeks after the completion of treatment in the golden hamster.

Treatment	Total mg/kg	Route	Percent Suppression
Vehicle (DMSO)	-	PO	-
BL09186 (Glucantime®)	416 208	IM	36 21
BN38070	832	PO	2
BN38089	832	PO	-23*
BN38098	832	PO	-5*
BN38105	832	PO	1
BN38114	832	PO	2
BN38123	832	PO	-7*

\* Negative percent suppression signifies a greater mean number of amastigotes/liver in the experimental group than in the vehicle control group.

PO: per os  
IM: intramuscular

**Table II.** Summary of the suppressive activity of selected compounds against *Leishmania Viannia braziliensis* one week and eight weeks after completion of treatment in the golden hamster.

Treatment	Total mg/kg	Route	Percent Suppression	
			1 week	8 weeks
Vehicle (DMSO)	-	PO	-	-
BL09186 (Glucantime®)	832 208	IM	62 39	26 16
BN38070	780	PO	26	3
BN38089	780	PO	16	3
BN38098	780	PO	10	6
BN38105	780	PO	-10*	-6*
BN38114	780	PO	10	-12*
BN38123	780	PO	-13*	-10*

\* Negative percent suppression signifies a larger mean lesion area in the experimental group than in the vehicle control group.

PO: per os  
IM: intramuscular

**Personnel Employed from this Cooperative Agreement**

<u>Name and Position</u>	<u>Percent Effort</u>	<u>Length of Employment</u>
Virginia B. Waits Research Coordinator II	50%	07/01/94 - 07/31/94
Virginia B. Waits Research Coordinator II	57%	08/01/94 - 08/31/94
Virginia B. Waits Research Coordinator II	100%	09/01/94 - 09/30/94
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**Bibliography of Published Work**

None

**Graduate Degrees Resulting from this Cooperative Agreement**

None

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