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ANTILEISHMANIAL ACTIVITY OF SELECTED COMPOUNDS IN DOGS EXPERIMENTALLY INFECTED WITH LEISHMANIA DONOVANI

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SUMMARY

Experimental infections of **Leishmania donovani** in both mongrel dogs and a parasite-free strain of beagle dogs were studied in an attempt to develop an animal model to test potential antileishmanial drugs. The studies were divided into two separate experiments. In Experiment I mongrel dogs were used to determine the effectiveness of known antileishmanial agents in reducing the number of liver parasites after the intravenous inoculation of 6×10^8 amastigotes/kg of the Khartoum strain of **Leishmania donovani.** The agents were administered twice daily, intramuscularly for five consecutive days beginning on the 21st day after inoculation of amastigotes. Experiment I also served to obtain preliminary information about an experimental compound, 8-(6-diethylaminohexylamino)-6-methoxy-4-methylquinoline designated WR 6 026, found to be highly effective against **L. donovani** infections of hamsters. Meglumine antimoniate (Glucantime(R)), a standard antimonial compound was also used. In Experiment II parasite-free beagle dogs were infected in the same manner; however, the treatment regimen of the meglumine antimoniate was reduced from five to four days, begun on the 12th rather than the 21st day, given by the intravenous route rather than the intramuscular route and the animals were killed two days after completion of the treatment regimen rather than four. Experiment II also was designed to compare the effectiveness of spleen derived as opposed to liver derived amastigotes. These studies showed that(1) the dog, either mongrel or parasite free beagle, is an acceptable model for the preclinical testing of potential antileishmanial drugs, (2) spleen derived amastigotes are superior to liver derived ones in producing the experimental infections, (3) either intravenous or intramuscular administration of these two test compounds is acceptable, (4) the treatment regimen of 5 days is no better than 4 days, (5) assessment of drug effectiveness may be accomplished at 2 days after completion of therapy, (6) the dose level employed did not consistently effect cures as determined by a culture method or by microscopic examination of livers and spleens, (7) the experimental drug WR 6 026 was superior to meglumine antimoniate in reducing the number of liver parasites after amastigote inoculation.

INTRODUCTION

The importance of the leishmaniases in human health throughout the world has been emphasized recently [4,7]. Drugs currently available for use in the treatment of these parasites, including sodium stibogluconate (Pentostam (R)) and meglumine antimoniate (Glucantime (R)),

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are not satisfactory. These drugs are relatively toxic and often not curative [4,9]. New and better drugs for treatment of the leishmaniases are therefore needed.

In an effort to identify new drugs for potential use in the treatment of leishmaniasis, the 8-day **in vivo** procedure of STAUBER et al. 1958, was modified to allow the weekly testing of 10 to 15 selected compounds for suppressive activity against **Leishmania donovani** in the golden hamster [4]. Using this procedure several new compounds active against **L. donovani** have been identified [6] and modifications of this procedure have been used to demonstrate dramatically the increased efficacy of compounds by encapsulation into liposomes [1,2].

Although the results obtained to date are promising, use of these new compounds in humans must await further study in hosts other than the hamster. The purpose of the studies reported herein were twofold: (1) to determine the usefulness of the dog as a test model in the testing of selected compounds identified in the primary screening procedure, (2) to obtain preliminary information regarding the effectiveness in the dog of a compound (WR 6 026) that was found in the hamster to be 700 times more effective than meglumine antimoniate, a compound used clinically today.

METHODS AND MATERIALS

Experiment I

Twenty young adult mongrel dogs (*) approximately 6-12 months of age and weighing 8.5-16.0 kg were apportioned into 6 groups. Two groups [those dogs receiving vehicle control (HEC-Tween) or 104 mg of Sb/kg a day as meglumine antimoniate] had 4 dogs each; the remaining 4 groups had 3 dogs each. Each group had 1 male; the remainder were females. Each animal was inoculated intravenously with amastigotes of the Khartoum strain of **L. donovani**. The amastigotes were derived from a combination of both the spleen and liver of infected hamsters. From infected dogs, percutaneous liver biopsies [3] were taken and the parasite densities quantitated at 7, 14 and 21 days after infection in an attempt to determine when the infection reached the optimum level for initiaton of therapy. The groups received respectively the drug vehicle (0.1% Tween 80 plus 0.5 hydroxyethyl cellulose designated HEC-Tween), 104 or 3.25 mg of Sb/kg a day (MKD) as meglumine antimoniate or 3.25, 0.8125 or 0.2031 MKD of 8-(6-diethylaminohexylamino)-6-methoxy-4-methylquinoline, designated WR 6 026. Therapy via the intramuscular route was initiated at 21 days postinfection and continued twice daily for 5 consecutive days. Four days after completion of therapy the dogs were killed and parasite densities in the livers were quantitated [8].

Amastigote suspension from the spleen or liver was prepared as described previously [5]. Briefly this consisted of mincing the heavily infected tissues in Minimum Essential Medium (MEM) in a Ten Broeck tissue grinder. The suspension was diluted to 1.2×10^8 amastigotes per ml. Five ml of spleen and liver suspension was injected intravenously into each dog. One day after completion of therapy each dog was weighed, killed and the liver removed and weighed. The ratio of the number of amastigotes per host liver nucleus was determined from liver impressions stained with Giemsa's stain and mean numbers of parasites per liver were calculated [8,10]. Tissues from livers of dogs which were found to be microscopically negative for **L. donovani** were cultured in Tanabe's medium [11]. Approximately 1 gram of liver was ground in 5 ml of Eagle's MEM, the 0.1 ml aliquots of this suspension were placed into each of 3 tubes of culture media. Employing a phase contrast microscope, cultures were examined for the presence of promastigotes for a period of 30 days at weekly intervals.

Experiment II

Nine male beagle puppies 10 weeks of age obtained from a parasite-free source (*) and weighing 2-3 kg were apportioned into groups of 3 and inoculated as described above with either spleen or liver derived amastigotes. At 12 days following the infection, each animal received intravenously (rather than intramuscularly as in Experiment I) one of the following twice daily doses for 4 consecutive days; Group 1 — vehicle control (HEC-Tween), Groups 2 & 3 — 104 or 13 MKD respectively of

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meglumine antimoniate. Two days after completion of therapy, the dogs were killed and liver parasite densities were determined [8,10]. Other procedures were as described for Experiment I, with the exception that no cultures of livers were done since impressions of either the liver or spleen of each beagle was positive for amastigotes on microscopic examination.

RESULTS AND DISCUSSION

In Table I are shown the results after treatment of mongrel dogs with meglumine antimoniate and the lepidine, WR 6 026. Meglumine antimoniate effected 82.7% and 42.9% suppression at 104 and 3.25 mg of Sb/kg/day respectively, whereas, WR 6 026 effected 93.9%, 83.7% and 68.1% suppression at much lower dose levels of 3.25, 0.8125 and 0.2031 MKD respectively. This superior effectiveness of WR 6 026 over the antimony containing compound although not as marked as observed in hamsters, nevertheless parallels those data [6]. Furthermore, all dogs which received the antimony bearing compound were positive for **L. donovani** as determined by the culture method employed. In contrast, three of nine animals receiving WR 6 026 were culture negative. Results from this experiment also indicated that the optimum time for demonstrating the effectiveness of a test compound was likely 12-14 days rather than at 21 days, the time of beginning the treatment of Experiment I animals.

TABLE I

Comparison of the suppressive effects of meglumine antimoniate and WR 6 026 on **Leishmania donovani** in the mongrel dog

Compounds	MKD(*)	% Wt. change	Number of dogs positive by culture	Mean number parasites/liver	Percent suppression
Vehicle control	—	+23.68	4/4	736,500,000	—
Meglumine Antimoniate	104	+17.07	4/4	127,500,000	82.69
	3.25	+23.53	3/3	576,670,000	42.86
WR 6 026	3.25	+30.77	1/3	45,000,000	93.89
	0.8125	+20.59	3/3	120,000,000	83.70
	0.2031	+39.29	2/3	235,330,000	68.05

(*) Meglumine antimoniate given in mg of Sb/kg body weight/day

In Table II are shown data from Experiment II which was designed to improve the animal model. Six differences should be noted in this experimental design as compared to that in Experiment I.

(1) Parasite-free beagles of a preferred single sex were readily available at a weanling age from commercial sources and were used rather than mongrel dogs of both sexes. It was anticipated that standardization of source, breed, sex, size and age of the dog would enhance the reproducibility of the experimental results. Although the beagle was a satisfactory standard model for experimental infections with **L. donovani**, other breeds of dogs may be as satisfactory or superior to the beagles and our laboratory is presently evaluating other breeds of dogs for this purpose.

(2) The time for beginning the drug treatment was reduced from 21 to 12 days in Experiment II on the basis of parasite evaluation of liver biopsies from the **L. donovani** infected mongrel dogs in Experiment I. There is a distinct possibility of altering this time interval as additional information is obtained from liver biopsies from infected dogs.

(3) The number of days of treatment was reduced from 5 to 4 thus conserving the amount of drug per experiment by 20%.

(4) The route of drug administration was changed from intramuscular to intravenous to compare the efficacy of these routes of administration of the test compounds in the canine. Both routes of administration were acceptable and paralleled previous results obtained in hamsters.

(5) Rather than mixing amastigotes from the spleens and livers of donor hamsters, amastigotes from each source were administered separately. Previous observations in our labora-

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tory had determined that amastigotes derived from hamster spleens resulted in higher infections of **L. donovani** in the hamster than did an equivalent number of amastigotes derived from liver. Control beagles receiving spleen derived amastigotes had considerably greater numbers of amastigotes in their liver than those receiving liver derived amastigotes. All dogs in Experiment II were positive for **L. donovani** by microscopic examination of impressions of liver or spleen. The observed 66.6% (spleen derived) and 100% (liver derived), the 48% (spleen derived) and 66.2% (liver derived) suppression for meglumine antimoniate at the two dosage levels employed roughly parallels the suppression observed in hamsters using this drug.

TABLE II

Comparison of the numbers of **Leishmania donovani** in beagle puppies receiving meglumine antimoniate and vehicle (HEC-Tween)

Compounds	Dog identification	Organ source of amastigotes	MKD(*)	% Wt. change	Mean No. amastigotes/liver(**)	Percent suppression
Vehicle Control	LB27	Spleen	—	+9.9	5,670,000,000	—
	MQ27	Liver	—	+7.6	136,000,000	—
	LO27					
Meglumine Antimoniate	LN27	Spleen	104	+2.8	377,600,000	66.6
	LI27	Liver	104	+9.5	—0—	100.0
	MI27					
	KI27	Spleen	13	+7.2	2,719,200,000	48.0
	KJ27	Liver	13	0.0	46,200,000	66.2
	LD27					

(*) Meglumine antimoniate given in mg of Sb/kg body weight/day

(**) Livers and/or spleens were positive for amastigotes on light microscopic examination; hence, no cultures were done.

(6) Dogs were killed two days after completion of treatment rather than four. This reduced total time of testing a drug against **L. donovani** in the canine model.

No evidence of drug toxicity was observed in any of the animals in either experiment. This determination was based upon macroscopic and microscopic examinations of liver lesions as well as clinical signs. The animals of Experiment II after receiving the concentrated suspension of amastigotes were listless and had pale mucous membranes suggesting an anaphylactic reaction. This reaction could likely be avoided by slower injection of the amastigote suspension; preferably with less tissue.

The results of these experiments suggest that: (1) the dog is an acceptable model for use in preclinical testing of various compounds against leishmaniasis, and (2) meglumine antimoniate is an effective compound against leishmaniasis in the dog but is less effective than that of WR 6 026.

RESUMO

Atividade anti-Leishmania donovani, de compostos selecionados, em cães infectados experimentalmente.

Estudaram-se infecções experimentais por **Leishmania donovani** em cães vira-latas e em uma linhagem de "beagles" parasitologicamente negativos, com o objetivo de desenvolver um modelo animal para ensaio de fármacos potencialmente ativos no calazar.

No Experimento I utilizaram-se cães vira-latas para determinar a eficácia de determinados compostos conhecidos, na redução numérica dos parasitas localizados no fígado, após injeção intra-venosa de 6 x 10^8 amastigotas/kg da linhagem Khartoum de **L. donovani.**

No Experimento II, cães de raça "beagle" foram infectados da mesma maneira, modificando-se apenas o esquema de administração de um dos quimioterápicos pesquisados (antimoniato de meglumina — Glucantime).

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Nossos estudos comprovaram que, ambas as variedades de cães constituem modelo experimental adequado para o tipo de ensaio realizado. A droga experimental WR 6026 mostrou-se superior ao antimoniato de meglumina.

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