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PRELIMINARY EFFICACY TRIAL OF CYMELARSAN IN MICE ARTIFICIALLY INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI* ISOLATED FROM A DOG IN ZAMBIA

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ABSTRACT

An efficacy trial of Cymelarsan® on a Zambian strain of *Trypanosoma brucei brucei* was done. Twenty-five male mice were infected intraperitoneally with 10^6 of *T. b. brucei* isolated from a dog. Five groups of 5 mice were treated with 0 (control), 0.25, 0.5, 1.0 and 2.0 mg/kg Cymelarsan, respectively. The target was to achieve aparasitaemia for 30 days post-treatment, euthanising those that remained parasitaemic or relapsed before then. The 0.25 and 0.5 mg/kg groups remained parasitaemic although the parasitaemic levels were reduced. The 1.0 mg/kg group had a proportion of aparasitaemic mice. However, all mice in the 2.0 mg/kg group remained aparasitaemic until day 20 when 2 mice relapsed. These resullts suggested that more than 2.0 mg/kg was required to eliminate this strain.

Key Words: Trypanosomiasis, Cymelarsan (Mel Cy), mice

The control of trypanosomiasis in prevalent countries has primarily depended upon the effective use of chemotherapy. Vaccination, which to date has attracted a lot of active research, has not been very successful due to the parasite's ability to vary its antigenic coat[5]. Furthemore, over a long period of time very few drugs have been developed against the parasite, resulting in strains resistant to the few drugs in use. *Trypanosoma brucei brucei (T. b. brucei)* is one of the common trypanosoma species in Zambia. The most commonly used drugs to control this and other trypanosome species in the country have mainly been diminazene aceturate (Berenil® ; Hoechst, S. Africa), and isometamidium chloride (Samorin® ; M&B, S. Africa). Although information on the levels of strains resistant to these drugs is limited in Zambia, the reported

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levels worldwide have indicated the need to search for alternative drugs.

Cymelarsan (Mel Cy. RM110; Rhone Merieux, France) was developed in 1985 specifically for veterinary use by Dr Friedheim from a combination of melarsenoxyde and cysteamine[9]. The drug, which is a trivalent arsenical and is currently being evaluated as a trypanocide, has been shown to have activity against the *Trypanozoon* subgenus to which *T. b. brucei* belongs in artificial and natural infections of camels, horses, cattle and buffaloes[2]. In camels artificially infected with *T. b. evansi* in Niger, the parasite disappeared from the blood for 63 days after treatment at dosages of 0.625–1.25 mg/kg[12]. Similar results were reported from Ethiopia in artificially infected camels[13]. In Kenya, however, of the naturally infected camels that were treated at dosages between 0.2–1.2 mg/kg one of them treated at 0.4 mg/kg relapsed[8]. It is not reported whether this was a case of re-infection or a genuine relapse of the original infection. The drug is available as a white powder highly soluble in water. It is administered either intramuscularly or subcutaneously.

The present study was carried out to observe the effect of Cymelarsan on a local (Zambian) strain of *T. b. brucei* and establish a dosage appropriate to treat cases for up to 30 days post infection (PI). Research to date has concertrated on *T. evansi* and little information is available on the other species in the *Trypanozoon* subgenus and the animal species they infect. This study also investigates these factors. The strain of *T. b. brucei* used was isolated from a dog in Chipata, the eastern province of Zambia and is infective to mice. The strain has been tested and found to be susceptible to Berenil at the normal curative dose of 3.5 mg/kg[6].

Twenty-five in-bred, white male mice weighing an average of 25 grams were used in the experiment. Mice were infected with *T. b. brucei* by inoculation of approximately 10^6 parasites intraperitoneally (I. P.). The mice were monitored for development of infection before treatment on days 3 and 8. They were then divided into treatment groups A, B, C, D and E, with A being the control while B, C, D and E received 0.25, 0.5, 1.0 and 2.0 mg/kg I. P. of Cymelarsan, respectively. This dosage range was arrived at from previous knowledge on an experiment carried out on *T. b. evansi* in which 2 mg/kg was the maximum dose required to cure early and chronic infection in mice[11]. After treatment, persistence of infection and/or relapse was monitored on days 1, 3, 6, 13, 16 and 20. It was intended to examine the mice twice weekly for 30 days post-treatment sacrificing those groups that tested positive. The dosage was certified curative if it caused aparasitaemia for 30 or more days. The parasitaemic levels before and after treatment were determined by direct wet blood film examination under a microscope at ×400 magnification[7]. These levels were recorded on the basis of log equivalent values (L. E. Vs) using the Lumsden matching technique.

The L. E. Vs of days 3 and 8 before treatment were on the average of 8.4 in all groups of mice. After treatment with Cymelarsan the 2 mg/kg group remained

aparasitaemic up to day 20 when two mice in the group tested positive and the whole group was euthanised. Aparasitaemia in the 1 and 2 mg/kg groups was noticed as early as 24 hours after treatment. The L. E. Vs in the 0.25 mg/kg group decreased after treatment but parasitaemia never disappeared completely, whereas the 0.5 and 1 mg/kg groups had a proportion of mice negative to infection up to day 16 when all groups showing infection were euthanised. The exact details of results in all groups after treatment are summarised in Table 1:

From the experiment, it was apparent that either a reduction in the levels of parasitaemia or complete aparasitaemia, indicating some form of drug effect, was obtained after administration of the drug. However, whether this was a trypanocidal or merely a transient suppressive effect could not conclusively be deduced from this experiment. It was also apparent from the experiment, as a result of the relapse observed after 20 days in the 2 mg/kg group, that to cure completely or suppress the parasite for 30 days a dose higher than 2 mg/kg was required. This differs from the minimum curative dose (M. C. D) required by mice infected with *T. b. evansi* shown in an earlier experiment[11], although the significance of this difference was not statistically tested. That the species difference was responsible for this observed difference is possible, however, the possibility of this local strain responding differently should equally be considered. An earlier report[3] in which *T. b. brucei* and *T. b. evansi* were tried together did not indicate the exact dose used to cure the infection of *T. b. brucei*, although it reported a similarlity in the requirement of a higher dose of the drug to cure chronic infections of both species. Therefore, to establish whether the difference in M. C. D between *T. b. brucei* and *T. b. evansi* is a result of species difference or merely a characteristic of this strain, more experiments should be carried out.

Another factor that could have influenced the higher dose requirement might have been the length of the postinfection period when treatment was effected. This is

Table 1. The number of aparasitaemic mice and the duration of aparasitaemia per group of mice exposed to different dosages of Cymelarsan.

Group	No.	Dosage mg/kg	Day :	No. of aparasitaemic mice per group 1	3	6	13	16	20
A *	5	0.0		0/5	0/5	0/5	0/5	0/5	0/5
B	5	0.25		0/5	0/5	1/5	0/5	0/5	—
C	5	0.5		1/5	4/5	5/5	0/5	1/5	—
D	5	1.0		4/5	4/5	5/5	2/5	2/5	—
E	5	2.0		5/5	5/5	5/5	5/5	5/5	3/5

* = Untreated control.
No. = Number of mice.
— = Euthanised.

especially so with *T. b. brucei*, which localises in the brain, a privileged site, where it is inaccessible to drugs or the concentration of the drug that penetrates the tissue is insufficient to be trypanocidal[4,5,10]. *T. b. brucei* being a cause of acute infections in mice, 8 days PI was a long enough period for it to establish itself in tissues and thus interfere with the M. C. D. This could further explain the relapses noticed in the 2 mg/kg group after 20 days as being relapses from the tissues. However, to what extent Cymelarsan is effective on tissue forms, if any, is a point that requires investigation.

Berenil® , to which Cymelarsan is known to exhibit cross-resistance[1], was found to cure this parasite at the normal dose of 3.5 mg/kg[6]. It is therefore, not possible that cross-resistance was responsible for the higher than normal dose required to treat the infection. Furthermore, Cymelarsan has not been used in this region before so direct resistance does not seem a credible reason either.

More research on the drug's availability, applicability and affordability for general practice would be especially valuable as the diseases it is meant to treat are prevalent in third world nations where money is an important limiting factor in the choice and use of drugs.

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