RESEARCH COMMUNICATION

Suggested dosage rates of melarsoprol in the treatment of mice experimentally infected with *Trypanosoma brucei gambiense*

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ABSTRACT

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One group of BALB/c mice infected with a highly virulent strain of *Trypanosoma brucei gambiense* were treated intraperitoneally with three series of three injections (each injection of 10 mg/kg) of Mel-B separated by seven days of rest, while a second group was treated once by a single injection All the Mel-B treated mice in both experiments were negative for parasites when examined using either the wet blood film or buffy coat methods, but were intermittently PCR positive during the sampling period. We encourage the use of a repeat negative PCR test over a one month period in combination with corroborative clinical and parasitological investigation to be suggestive of cure in experimental animals previously infected with trypanosomosis. In view of the exorbitant costs of Mel-B and its extreme toxicity, we recommend that Mel-B be given as one course of two injections (each equivalent to 10 mg/kg) separated by 2 d of rest in experimentally infected rodent models.

Keywords: Mel-B, mice, PCR, *Trypanosoma brucei gambiense*

INTRODUCTION

Only four drugs are currently in use for the treatment of human sleeping sickness caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* infections. Suramin and Pentamidine are used to treat the early stages of the disease, the former being used against *T.b. rhodesiense* and the latter against *T.b. gambiense* infections. Only two drugs are available to treat the late stage of the disease when trypanosomes have invaded the central nervous system: melarsoprol (Mel-B) and DL-alpha-difluoromethyornithine (DFMO). Melarsoprol is the drug of choice in the treatment of both *T.b. gambiense* and *T.b. rhodesiense* infections. It is an arsenical compound, and is associated with severe side effects in 20% of treated patients (Pepin, Milord, Guerin, Mpia, Ethier & Mansinsa 1989). In addition, relapse cases have been reported following Mel-B treatment, though the reasons for these are unknown. Melarsoprol is relatively toxic and produces encephalopathic reactions which may be fatal (WHO, 1998). Nevertheless, with attention to detail, these toxic effects can be minimized. The drug is issued in ampoules, ready for use, as a 3,6% solution in propylene glycol. Melarsoprol must never be used prior to a preliminary course of suramin which is administered to clear the patient's blood of trypanosomes in order to avoid a Jarisch-Herxheimer reaction which occurs when large numbers of trypanosomes are killed (Bryceson 1976). The usual dose of melarsoprol recommended for each administration is 3,6 mg/kg body weight, the maximum single dose being 5 mℓ. One such administration is given on each of 3 or 4 consecutive days and, after a rest period of one

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week, the course is repeated. A third course of three or four injections may be given after a further rest period of 1 week. In this study, the Mel-B dose required to clear an infection in mice experimentally infected with a virulent strain of *T.b. gambiense* was examined, and a possible yardstick of assessment of cure is given.

MATERIALS AND METHODS

Two experiments were devised. In the first, the treatment schedule of mice experimentally infected with trypanosomes closely mimicked that given to humans suffering from sleeping sickness (i.e. three series of three injections separated by 7 d of rest). In brief, ten 5-week-old male inbred BALB/c mice each were individually infected with 5 x 10^3 blood stream-forms of *T.b. gambiense* IL3707 intraperitoneally (ip) in 0,5 mℓ of PSG(+) (phosphate, sodium, glucose) buffer. This parasite is a highly virulent strain, as has been described by Inoue, Narumi, Mbati, Hirumi, Situakibanza & Hirumi (1998). The mice were then separated into two equal groups which were labeled as experimental and control groups, respectively. At 4 d post-infection (DPI), the mice in the experimental group were started on a treatment regimen which consisted of Mel-B (obtained from WHO, Geneva) administered ip at the dosage rate of 10 mg/kg/day (Poltera, Hochmann & Lambert 1981) and comprised three series of three injections each (one injection per day on 3 consecutive days) separated by 7 d of rest. In the control group, PSG(+) buffer was administered using a similar protocol. In the second experiment, ten 5-week-old male BALB/c mice were similarly infected and divided into two equal groups: experimental and control. At 4 DPI, the mice in the experimental group were treated once with a single ip injection of Mel-B at the dose rate of 10 mg/kg. The mice in both experiments were examined periodically (see below) for the appearance of parasites in peripheral blood by wet blood film and buffy coat scrutiny, measurement of packed cell volume (PCV), and PCR.

Parasitological diagnosis

The diagnostic examinations of the mice in the experimental group in the first experiment were performed as follows: 4 DPI (just before the start of the first schedule of treatment); 7 DPI (one day after the first schedule of treatment); 14 DPI (just before the second schedule of treatment); 17 DPI (a day after the second schedule of treatment); 24 DPI (just before the third and last schedule of treatment); and 27 DPI (a day after the last schedule of treatment). Thereafter, the diagnostic examinations were conducted at random time intervals up to 104 DPI. The mice in the experimental group in the second experiment were observed at random time intervals for evidence of infection 1 d after treatment, and for up to 61 DPI. Diagnostic examination of the control groups of mice in both experiments were performed at the same intervals as the experimental groups until they died.

DNA extraction from small blood samples

About 10 µℓ of blood from the tail of each mouse on each occasion that diagnostic tests were performed, was pipetted into 100 µℓ of lysis buffer containing a final concentration of 10 mM Tris HCl (pH 8,0), 10 mM EDTA (pH 8,0), 100 mM NaCL, 0,5% SDS and 300 µg/mℓ of proteinase K. After incubation for 2 h S at 55 °C (Eyla, Dry Thermobath MG-1100), 100 µℓ of a phenol:chloroform (1:1) solution was added and thoroughly mixed with a Vortex machine. The samples were then centrifuged at 4 000 g for 5 min at room temperature and the supernatant (containing DNA) transferred to a new tube. This phenol:chloroform extraction procedure was repeated twice. Ten µℓ of 3 M sodium acetate (pH 5,2) and 250 µℓ of 100% ethanol was added to the supernatant and frozen at –80 °C for 30 min. These samples were subsequently thawed at room temperature. After centrifugation at 4 000 g for 15 min at 4 °C, the supernatant was removed, and 500 µℓ of 70% ethanol was added to the DNA pellet. The samples were again centrifuged at 4 000 g for 5 min at 4 °C and the supernatant removed. The DNA samples in the micro-tubes were then air-dried (Eyela, Centrifugal Vaporizer). Each DNA sample was dissolved in 10 µℓ of Tris-EDTA buffer and kept frozen at 30 °C until used.

PCR of VSG 117 genes

DNA preparations were amplified in a final volume of 30 µℓ in a Perkin Elmer PCR machine (Gene Amp 2400). In preparation of the master mix, the following volumes were used: 20,4 µℓ DDW; 3 µℓ of 10 x PCR buffer (Perkin Elmer); 3 µℓ of 2 mM dNTPs (Perkin Elmer), 0,3 µℓ of VSG 117 primer (50 pmol/µℓ each of sense and antisense) (Bromidge, Gibson, Hudson & Dukes 1993), and 0,3 µℓ Taq (*Thermus aquaticus*) DNA polymerase (Perkin Elmer). Three microlitres of DNA sample was used as template per reaction programme. In the PCR reaction programme, samples were incubated at 94 °C for 10 min in an initial denaturation step, followed by 40 cycles at 94 °C (30 s), 51 °C (1 min) and 72 °C (2 min). The PCR reaction products were analysed by agarose gel electrophoresis and detected visually by UV transillumination (Atta, Bioinstrument) of the ethidium bromide-stained gel.

RESULTS

Summaries of results of the diagnostic tests of the mice in the experimental group in the first and second experiments are shown in Tables 1 and 2,

TABLE 1 Results obtained in Experiment 1 of various diagnostic tests used to detect *Trypanosoma brucei gambiense* (IL3707) in BALB/c treated with either Mel-B or PSG(+)

Test	Group	Days post-infection											
		4	7	14	17	24	27	41	53	67	74	80	88	104
Tail blood	PSG(+)	4/5[a]	1/1	[b]										
	Mel-B	4/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Buffy coat	PSG(+)	4/5	1/1	[b]										
	Mel-B	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
PCR	PSG(+)	4/5	1/1	[b]										
	Mel-B	5/5	1/5	0/5	0/5	0/5	0/5	ND	2/5	3/5	1/5	3/5	0/5	ND

[a] Results are shown as the proportion: $\frac{\text{Number of mice in which the presence of parasites was detected}}{\text{Number of mice tested}}$

[b] All mice dead

ND: Not done

TABLE 2 Results obtained in Experiment 2 of various diagnostic tests used to detect *Trypanosoma brucei gambiense* (IL3707) in BALB/c treated with either Mel-B or PSG(+)

Test	Group	Days post-infection					
		4	5	26	41	47	61
Tail blood	PSG(+)	4/5[a]	5/5	[b]	-	-	-
	Mel-B	5/5	0/5	0/5	0/5	0/5	0/5
Buffy coat	PSG(+)	5/5	5/5	[b]	-	-	-
	Mel-B	5/5	0/5	0/5	0/5	0/5	0/5
PCR	PSG(+)	5/5	5/5	[b]	-	-	-
	Mel-B	5/5	0/5	0/5	2/5	0/5	0/5

[a] Results are shown as the proportion: $\frac{\text{Number of mice in which the presence of parasites was detected}}{\text{Number of mice tested}}$

[b] All mice dead

respectively. In both treatment schedules, the mice revealed no trace of parasites 24 h after treatment when examined by parasitological methods and PCR. Untreated mice from both experiments were dead by 10 DPI, all revealing heavy parasite burdens. Mice treated with either a single dose or three courses of Mel-B were negative for parasites when examined using both the wet blood film and buffy coat techniques upto the end of the experiment at 61 or 104 DPI respectively, but were intermittently PCR positive during the sampling period. No toxic effects resulting from the Mel-B therapy was observed in any of the treated mice which were physically healthy with normal PCV's of around 56% from approximately the second week of treatment and throughout the rest of the experimental period. No relapses to the treatment were observed.

DISCUSSION

Trypanosoma b. gambiense IL3707 is a highly virulent parasite in BALB/c (Inoue *et al.* 1998). Most of control (PSG-treated) mice had died by about the 7th DPI. There was an excellent correlation between the PCR and parasitological methods of diagnosis at both 4 DPI and 5 DPI in the groups of experimental animals where all parasitologically positive mice were also PCR positive. The most significant observation

is that at about 24 h after a single administration comprising one injection of Mel-B at a dosage rate of 10 mg/kg, all the mice were both parasitologically and PCR negative, demonstrating the efficacy of S Mel-B. Parasitological methods of diagnosis of whole blood and buffy coat of all the Mel-B treated mice were negative until the end of the observation periods.

Various techniques used in the diagnosis of trypanosomosis, such as the parasitological methods and PCV, and the highly sensitive and specific PCR which were used in this study are all useful tests. We consider that a repeated negative PCR test during a 1 month period, in combination with corroborative clinical and parasitological methods of diagnosis, is suggestive of cure. In view of the exorbitant costs of Mel-B and its extreme toxicity, we recommend that Mel-B, given as one course of two injections (each equivalent to 10 mg/kg) separated by 2 d of rest is adequate to cure rodents suffering from *T.b. gambiense* in laboratory experimental studies.

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