

IVERMECTIN AND DIETHYLCARBAMAZINE TRIALS IN LEAF MONKEYS (*PRESBYTIS CRISTATUS*) INFECTED WITH *WUCHERERIA KALIMANTANI*

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Abstract. Clinical trials of Ivermectin in single oral doses of 200, 400 and 1,000 mg/kg body weight or in multiple doses of 200 mg/kg body weight for 5 consecutive days were performed in leaf monkeys (*Presbytis cristatus*) infected with *Wuchereria kalimantani*. Optimal microfilaricidal effect occurred at 200 mg/kg body weight. The drug was less effective than diethylcarbamazine in this animal model for human filariasis but had no adverse effects.

INTRODUCTION

Lymphatic filariasis is still a major public health problem in many tropical and subtropical countries. More than 90 million people are currently infected with these parasites (WHO, 1984). Of the three species infecting man, *Wuchereria bancrofti* is the predominant parasite.

Attempts to establish an animal model for *W. bancrofti* had been relatively unsuccessful (Cross *et al*, 1979; Dissanaik and Mak, 1975). Microfilaremia is transient and the density is low. In contrast *W. kalimantani*, the only other species in the genus *Wuchereria*, is a naturally occurring lymphatic filarial parasite of the leaf monkey (*Presbytis cristatus*) (Palmieri *et al*, 1980). The infection is long-lasting and the microfilarial density is high, making it a suitable animal model for drug trials (Palmieri *et al*, 1983).

This study was conducted to evaluate the micro- and macrofilaricidal effects of Ivermectin and diethylcarbamazine (DEC) in leaf monkeys, infected with *W. kalimantani*.

MATERIALS AND METHODS

The study was conducted in 3 phases :

First phase

Twelve leaf monkeys (*Presbytis cristatus*),

naturally infected with *W. kalimantani* were divided into two groups according to their body weight, sex and level of microfilariae. They were then randomly allocated into groups A or B (Table 1).

Each animal was blindly treated with a single oral dose of 200 µg per kg body weight of either Ivermectin or placebo, after an overnight fast. The drug was dissolved in 5 ml sterile distilled water in a disposable syringe and administered orally through a disposable stomach tube. The stomach tube was carefully installed through the nose to three quarters of its length while closely observing the animal. When the tube reached the stomach, a characteristic acid smell was observed. The drug in the syringe was repeatedly rinsed with sterile distilled water (15 ml total volume). No food or water were given in the first three to four hours after treatment. Each animal was clinically observed after the treatment for its daily activities and feeding habits. Microfilaremia (Mf) densities were monitored the night before treatment and weekly post-treatment till week 28. The night blood was taken sequentially starting with the first animal and ending with the last one, each time in the same fashion, during the night at approximately the same time. Body weight, hemoglobin level, WBC and eosinophil counts were monitored monthly for 4 months.

By the end of the study, one monkey from each group was sacrificed to assess the macrofilaricidal effect of Ivermectin.

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Table 1

The mf densities, sex and body weight of the leaf monkeys prior to the treatment with Ivermectin or placebo.

Group A				Group B			
No.	Sex	Weight (kg)	Mf count (/20 µl)	No.	Sex	Weight (kg)	Mf count (/20 µl)
18	M	4.0	243	04	F	3.5	268
20	M	4.0	169	31	M	3.0	184
19	F	4.5	169	53	F	4.0	49
32	F	3.5	20	52	F	4.0	63
09	F	3.5	52	51	M	6.0	26
33	F	3.0	8	08	F	4.0	8

Table 2

The characteristics of monkeys included into a stratified randomized double blind trial of 12 mg DEC/kg for 12 days or placebo.

Group	No.	Sex	Weight (kg)	Mf density /20µl/0.5ml	WBC	Eos
DEC Treated Group	32	F	3.5	8/69	8,615	12
	19	F	4.0	26/606	8,420	12
	04	F	3.5	69/807	9,105	12
	53	F	3.0	38/269	10,145	10
	51	M	5.5	58/1,340	7,280	12
Group Control	09	F	3.5	15/178	9,555	8
	32	F	3.5	38/927	9,155	25
	18	M	4.0	58/1,242	6,915	11
	08	F	4.0	26/325	6,130	16
	31	M	3.0	127/1,059	6,365	8

Second phase

In the second phase, 10 monkeys were divided into two groups according to their body weight, sex and mf counts (Table 2). They were then randomly allocated either into a treatment group with DEC or placebo. The treatment group received 12 mg DEC per kg body weight for 12 consecutive days. The drug was dissolved in sterile physiologic saline solution and administered orally through a stomach tube as described earlier. The control group received sterile physiologic saline solution in the same volume as the treated group. Clinical parameters were monitored daily for 14 days.

Laboratory parameters were measured before and two, seven and twelve days after treatment. The mf counts were monitored similarly on a weekly basis for 24 weeks. At the end of the study, the DEC treated monkeys were sacrificed to evaluate the macrofilaricidal effect of DEC.

Third phase

The third phase was a randomized stratified trial involving 14 laboratory infected monkeys with *W. kalimantani* to evaluate the efficacy and safety of 3 different dose regimens of Ivermectin. Seven animals with high mf counts (> 50 / 20 µl

Table 3

The characteristics of monkeys included into a stratified randomized double blind clinical trial of high and multiple doses of Ivermectin.

Group	No	Sex	Weight (kg)	Mf density (/20 µl)	WBC	Eos	Dose (µg)
C	58	M	4.5	86	6,570	7	400,sd*
C	07	F	4.0	174	5,570	12	400,sd
C	23	F	4.0	28	12,615	11	400,sd
C	55	F	4.5	9	8,595	11	400,sd
D	30	F	4.0	332	8,475	12	1,000,sd
D	29	M	5.0	83	6,985	10	1,000,sd
D	27	F	5.5	27	7,270	12	1,000,sd
D	63	F	3.0	20	8,895	10	1,000,sd
E	34	F	4.0	126	9,960	13	200,md#
E	28	F	5.0	72	9,010	9	200,md
E	41	F	4.5	12	8,105	11	200,md
E	72	M	5.5	47	6,780	5	200,md
F	02	M	5.5	72	7,945	9	-
F	71	F	4.0	16	9,820	10	-

* sd = single dose

md = multiple doses

blood) and another seven with low mf counts (< 50 mf / 20 µl blood) were randomly allocated into 3 treatment groups (C, D, E) and one control group (F). There were 4 monkeys in the treatment group and 2 monkeys in the control group (Table 3). Treatment group C received a single oral dose of 400 µg Ivermectin per kg body weight, group D received a single oral dose of 1,000 µg Ivermectin per kg body weight and group E received 200 µg Ivermectin per kg body weight for 5 consecutive days. The drug was suspended in aquabidest. The control group received aquabidest only. Microfilarial counts were done as described above, till 12 weeks post-treatment. The behavior, food intake and characteristics of the excreta were monitored daily up to 14 days post medication. Rectal temperature was taken before, one and five days after the start of the treatment. Laboratory parameters consisting of serum biochemistry (alkaline phosphatase, SGPT, SGOT) were determined before, one day and five days after the first medication.

RESULTS

First phase

The mf densities decreased markedly after the first post treatment week and remained so for 4 weeks (Fig 1) in the treatment group. However, the densities increased gradually and were similar to the control group on the 25th week post treatment. Laboratory and clinical adverse reactions were insignificant. At autopsy, living and active adult worms were recovered (Table 4).

Second phase

In the second phase, the microfilaricidal effects of DEC were dramatic. The mf densities decreased to less than 10 mf/0.5 ml of blood, after the first post-treatment week and remained so for the entire 24 weeks observation period (Fig 2). No adult worms were recovered from any of the DEC treated monkeys. There were no significant laboratory or clinical adverse reactions.

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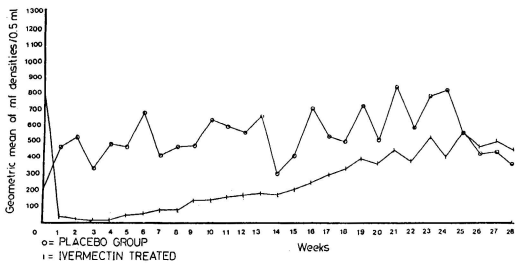


Fig 1—The microfilaricidal effect of a single oral dose of 200 µg Ivermectin in leaf monkeys infected with *W. kalimantani*.

Table 4

The macrofilaricidal effects of a single oral dose of 200 µg Ivermectin per kg body weight in leaf monkeys infected with *W. kalimantani*.

Monkey no.	No. of live worms recovered
20	5 females and 4 males
52	1 female and 1 male

Third phase

The microfilaricidal effect of Ivermectin in high single doses (400 µg or 1,000 µg/kg body weight) (Fig 3, 4) or multiple doses (5 × 200 µg/kg body weight) (Fig 5) were similar to the standard 200 µg/kg dose (Fig 1). The mf densities decreased temporarily after the first few weeks post-treatment and increased gradually until the 7-8 week post treatment when the mf densities

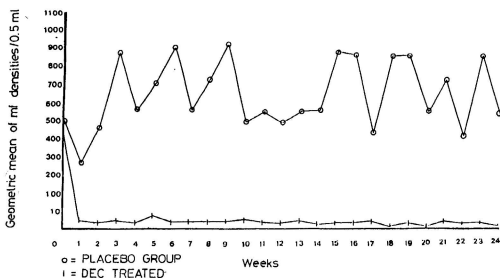


Fig 2—The microfilaricidal effect of diethylcarbamazine in leaf monkeys infected with *W. kalimantani*.

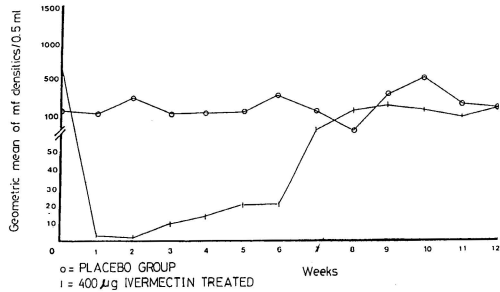


Fig 3—The microfilaricidal effect of a single oral dose of 400 µg Ivermectin in leaf monkeys infected with *W. kalimantani*.

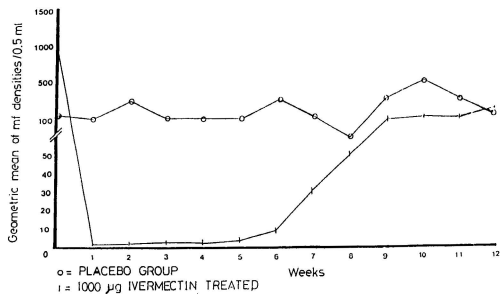


Fig 4—The microfilaricidal effect of a single oral dose of 1,000 µg Ivermectin in leaf monkeys infected with *W. kalimantani*.

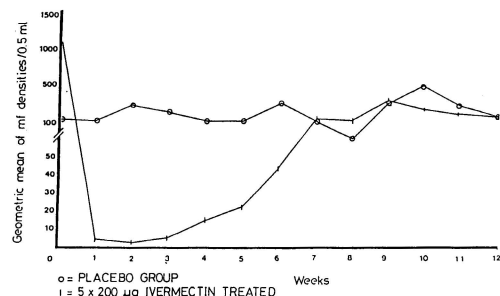


Fig 5—The microfilaricidal effect of multiple doses of 200 µg of Ivermectin in leaf monkeys infected with *W. kalimantani*.

reached pre-treatment levels of the control group. Clinical and laboratory adverse reactions were also insignificant.

DISCUSSION

Clinical trials of Ivermectin in single oral doses of 200 µg, 400 µg and 1,000 µg/kg body weight or in multiple doses of 200 µg/kg body weight for 5 consecutive days have been performed in leaf monkeys infected with *W. kalimantani*. The drug is a potent microfilaricide, decreasing the mf densities dramatically at one week post treatment. The microfilaricidal effect lasted for approximately 4 weeks, thereafter the mf densities increased gradually and reached pre-treatment levels in 7-24 weeks post-treatment. There was an indication that higher doses of Ivermectin (more than 200 µg/kg body weight) or multiple doses did not increase its microfilaricidal effects. It appeared that an optimal response was achieved at 200 µg/kg body weight and that higher doses seemed to be less effective. In comparison with diethylcarbamazine, the drug is less effective in this animal model. DEC is a potent micro- and macrofilaricide. Its microfilaricidal effects lasted during the entire study period of 24 weeks and at autopsy, no adult worms were recovered.

There were no significant adverse reactions observed at all doses of Ivermectin tested as well as with DEC. We conclude that Ivermectin is a microfilaricide against *W. kalimantani* and is less potent than DEC. It has no macrofilaricidal effects as has been reported in other studies (Mak *et al*, 1988, 1991; Kumaraswami *et al*, 1988; Campbell and Blair, 1978; Taylor and Greene, 1989).

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