THE EFFICACY OF A SINGLE-ORAL-DOSE ADMINISTRATION OF IVERMECTIN AND DIETHYLCARBAMAZINE ON THE TREATMENT OF FELINE *BRUGIA MALAYI*

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Abstract. The combination of ivermectin and diethylcarbamazine (DEC) have been shown to be superior to either drug alone for the suppression of *Brugia malayi* in humans, but their efficacy against infection with *B. malayi* in cats has never been investigated. Fourteen asymptomatic microfilaremic (1-200 microfilariae/20 µl blood) cats received oral doses of ivermectin (400 µg/kg body weight) and DEC (6 mg/kg body weight) as a single treatment. A two-month post-treatment examination revealed that 87-100% of the microfilariae in each subject had been cleared, with two of the subjects being amicrofilaremic. A further reduction in microfilarial levels was observed until the final follow-up, at 8 months post-treatment, when the mean clearance rate was 99% and 12 out of the 14 subjects (86%) were amicrofilaremic. The combination of ivermectin and DEC demonstrated a microfilaricidal effect superior to that of either drug used alone, both in the initial rapid clearance of microfilariae, and in sustaining the effect for 8 months. This finding has important implications for the control of brugian lymphatic filariasis in the cat reservoir.

INTRODUCTION

The World Health Organization has targeted the year 2020 for the eradication of lymphatic filariasis (Ottensen, 1998). Although 90% of the cases reported worldwide are caused by *Wuchereria bancrofti*, a further 10% are attributed to brugian filariasis, and are restricted to the Asian region (WHO, 1994; Micheal and Bundy, 1997). Subperiodic *Brugia malayi* has been reported in domestic cats from southern Thailand (Chansiri *et al*, 2002). This makes it difficult to control and eliminate the parasites in the endemic area. The drug that is currently used to treat brugian filariasis, diethylcarbamazine citrate or DEC, is unsatisfactory due to its requirement of daily oral intake for two to three weeks to achieve a maximal microfilaricidal effect (Piessens *et al*, 1981; Ewert *et al*, 1983a,b). However, DEC is the only drug that is effective for killing Brugian adult worms (Ewert and Emerson, 1979).

Ivermectin, a semisynthetic macrolide antibiotic, has a wide helminthicidal spectrum for parasites of animals (Campbell *et al*, 1983). It is effective for the clearance of microfilaria within 30-60 minutes, but cannot eliminate adult worms. Hence, microfilaria can recur within 1-2 months. Generally, ivermectin has been recommended for once-yearly treatment of onchocerciasis at a dose of 200 µg/kg body weight co-administered with either DEC (6 mg/kg body weight) or albendazole (400 mg) (Ismail *et al*, 2001). The efficacy of single dose combinations of albendazole, ivermectin and diethylcarbamazine for the treatment of human brugian and bancroftian filariasis have been reported (Aikat and Das, 1976, Moulia-Pelat *et al*, 1994, 1995;

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Ismail *et al*, 1998; Shenoy *et al*, 1998 a, b, 1999, 2000; Horton *et al*, 2000). However, their efficacy against infections with *B. malayi* in the cat reservoir has never been investigated. This study was designed as a comprehensive trial of the co-administration of ivermectin and DEC, to test their effectiveness in the treatment of domestic cats with microfilariamic and adults brugian filariasis.

MATERIALS AND METHODS

Animals

Fourteen *B. malayi* infected adult male and female domestic cats from Narathiwat Province were investigated and diagnosed for the type of infection. Identification of *B. malayi* infection (1 to 200 microfilariae per 20 microliters of blood) was carried out using the morphological characteristics, the localization of acid phosphatase stained blood film (Aoki *et al*, 1976) and PCR-based methods.

Assessment of microfilaremia

Cats were determined for their microfilarial periodicity by collecting blood at 2-hours interval for 24 hours. Twenty microliters of fresh blood was collected from an ear vein. Two duplicate lines of thick-blood smears were immediately made on a clean slide glass. The microfilariae were counted under a light microscope after the Giemsa stained blood film and microfilarial periodicity were further analyzed by the method of Aikat and Das (1976).

Treatment protocol

The infected cats were classified into 4 groups depending on the density of the microfilaria (Table 1). The *B. malayi* infected cats were treated with oral ivermectin and DEC at the dosages of 400 µg and 6 mg/kg of body weight, respectively. Blood samples were collected from ear veins at 9:00 pm on the day before and after treatment. The blood-circulating microfilariae assessment was performed as described above for 8 months.

Blood collection

The blood specimens were collected at the time of peak parasitemia (09:00-12:00 pm). Twenty microliters of ear-pricked blood was collected and transferred to a 0.5ml tube containing 25 µl of 0.002% SDS. The mixture was gently mixed and boiled at 95ºC for 15 minutes prior to centrifugation at 12,000 rpm for 10 minutes. PCR analysis was then performed on the supernatant.

Standard human *B. malayi* and feline *B. pahangi* were obtained from infected subjects and experimentally infected into cats. The periodicity of the parasites and the acid phosphatase stained blood films were tested to confirm the parasite species.

PCR amplification

PCR reaction amplification of the H*ha* I repetitive region of *B. malayi* was achieved using primers Bm1 (5′ GCGCATAAATTCATCAGCAA 3′) and Bm2 (5′ ATGACAACTCAATACTCGAC 3′) in a 25 µl mixture containing 1x PCR buffer, 0.1 mM dNTP, 1.5 mM $MgCl_2$ and 1 unit of *Taq* polymerase. The PCR mixtures were heated at 95ºC for 4 minutes prior to the PCR cycle. One cycle of PCR consisted of denaturation at 95ºC for 60 seconds, annealing at 55ºC for 60 seconds and polymerization at 72ºC for 90 seconds. The procedure was performed for an additional 29 cycles. The PCR product of 294 bp was amplified from *Brugia* sp. The PCR products were separated using 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized under a UV transluminator.

Traditional staining

Giemsa-stained thick blood films were performed according to standard methods.

Statistical analysis

Statistical evaluation was determined by the Student's *t* test using SPSS/PC$^+$ program.

Infectivity index

The infectivity index indicates the ability of transmission that was related to the number of microfilaria in a certain amount of blood. (Manubu, 1976; Park, 1988) It was determined using the following equation;

$$P = 1 - e^{-m} \quad \ldots\ldots\ldots(1)$$

"P" represents the probability of transmission and "m" represents the number of microfilaria per 20 µl blood.

Infectivity index (%) = (P) x 100

Table 1
Microfilaria (Mf) in 20 µl blood pre- and post-treatment with DEC and ivermectin examined using thick blood film Geimsa staining (GS) and PCR techniques.

Group	Number of cats	Mf density	#Cat	Pre-treatment		Post-treatment detection using thick blood film Giemsa staining (GS) and PCR															
						1 month		2 months		3 months		4 months		5 months		6 months		7 months		8 months	
				GS (%)	PCR	GS (%)	PCR	GS (%)	PCR	GS (%)	PCR	GS (%)	PCR	GS (%)	PCR	GS (%)	PCR	GS (%)	PCR	GS (%)	PCR
1	3	1-20	1	9 (100)	+	1 (11.1)	+	0.5 (5.5)	+	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
			2	13.5 (100)	+	1 (7.4)	+	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
			3	18.5 (100)	+	12 (64.8)	+	13.5 (72.9)	+	3.5 (18.9)	+	0.5 (2.7)	+	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
2	4	21-50	4	24.5 (100)	+	6 (24.5)	+	3 (12.2)	+	2.5 (10.2)	+	1.5 (6.1)	+	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
			5	64.5 (100)	+	28 (43.4)	+	4 (6.2)	+	2 (3.1)	+	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
			6	35.5 (100)	+	14.5 (40.8)	+	10.5 (29.6)	+	6.5 (18.3)	+	5.5 (15.5)	+	3 (8.5)	+	0 (0)	-	0 (0)	-	0 (0)	-
			7	40 (100)	+	4 (10.0)	+	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
3	3	51-100	8	58 (100)	+	5 (8.6)	+	2.5 (4.3)	+	1 (1.7)	+	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
			9	76 (100)	+	23 (30.3)	+	3.5 (4.6)	+	5 (6.5)	+	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
			10	95 (100)	+	30.5 (40.1)	+	20.5 (21.6)	+	4 (4.2)	+	5.0 (5.2)	+	6 (6.3)	+	0.5 (0.5)	+	0.5 (0.5)	+	0 (0)	-
4	4	151-200	11	167 (100)	+	15.5 (9.3)	+	13 (7.8)	+	15.5 (9.2)	+	12 (7.1)	+	12.5 (7.5)	+	5.5 (3.3)	+	0 (0)	-	0 (0)	-
			12	171 (100)	+	12 (7.0)	+	1.5 (0.9)	+	5 (2.9)	+	2 (1.2)	+	0 (0)	-	0 (0)	-	0 (0)	-	0 (0)	-
			13	171 (100)	+	13 (7.6)	+	11.5 (6.7)	+	17 (9.9)	+	18 (10.5)	+	17.5 (10.2)	+	13 (7.6)	+	13 (7.6)	+	10 (5.8)	+
			14	179 (100)	+	11 (6.1)	+	9.5 (5.3)	+	6.5 (3.6)	+	6 (3.3)	+	5 (2.8)	+	8.5 (4.7)	+	9.5 (5.3)	+	8 (4.5)	+
Mean number of microfilaria				80.2 (100)		12.9 (22.2)		6.7 (12.7)		4.9 (6.3)		3.6 (3.7)		2.7 (2.5)		2.0 (1.2)		1.6 (1.0)		1.3 (0.7)	
Probability of transmission (P)				1.0000		0.9999		0.9987		0.9926		0.9726		0.9328		0.8647		0.7981		0.7275	
Infectivity index (%)				100		100		99.9		99.3		97.3		93.3		86.5		79.8		72.7	

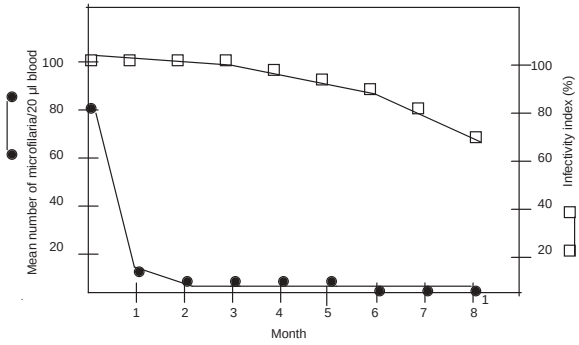

Fig 1–Mean blood circulating microfilarial density and infectivity index of pre- and post-treatment with a single-dose-oral administration of ivermectin and DEC.

RESULTS

After a single-oral-dose of ivermectin and diethylcarbamazine was used, rapid suppression of microfilaremia was achieved within 30 days (Table 1). The microfilarial density of all treatment groups dropped continuously for 30 days. The microfilaria in 9 of 10 cats in groups 1, 2 and 3 (low and medium microfilaria density between 1-100 mf) had complete clearance by 6 months.

The infectivity index was determined as the indication of the ability of transmission. It is shown in Fig 1 that the ability of microfilaria transmission decreased after drug treatment, suggesting that the possibility of transmission was decreased.

DISCUSSION

Our data (Table 1) indicate that the dosage is sufficient for mass chemoprophylaxis in *B. malayi* infected cats which remain as potential transmission reservoirs. Incomplete clearance of the parasite was observed in the cats in group 4 (microfilaria density > 100 mf). This result is similar to that of Shenoy and colleques (1998b) where a single-dose treatment with ivermectin (400 µg/kg) and DEC (10 mg/kg) against human brugian filariasis had a sustained effect for 1 year. They also demonstrated that the subjects in the two groups given DEC [ivermectin (200 µg/kg) with DEC (6 mg/kg) and DEC (6 mg/kg) with albendazole (400 mg)] had less intense microfilaremia 1 year after re-treatment than those given ivermectin with albendazole [ivermectin (200 µg/kg) with albendazole (400 mg)] (Shenoy *et al*, 2000). When the efficacy of the combination doses of ivermectin, DEC and albendazole were compared, it was reported that albendazole alone had no effect on the microfilarial levels at 1 year, whereas 47-64% of those given DEC and albendazole, and 14% of those given ivermectin with albendazole, were amicrofilaremic 1 year post-treatment (Shenoy *et al*, 1998a). Another study revealed that albendazole 400 mg with ivermectin 200 µg/kg, albendazole 400 mg with DEC 6 mg/kg and albendazole 600 mg with ivermectin 400 µg/kg could significantly reduced mf counts, with the albendazole-DEC-treated group showing the lowest mf levels at 18 and 24 months post-treatment (Shenoy *et al*, 1999).

In conclusion, a single-oral-dose of ivermectin and DEC was effective for sustaining the clearance of microfilariae in the cat reservoir for up to 8 months or more. To achieve effective suppression, a repeat dose every 8-12 months should be suitable for the prevention of disease transmission.

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