Microfilaricidal Efficacy of Ivermectin on Zoonostic *Brugia malayi* in Naturally Infected Cats

Sirichai Phantana[1], Nopporn Sarataphan[2], Gaysorn Chansiri[3], Kosum Chansiri[4]

[1]*Filariasis Division, Ministry of Public Health, Nonthaburi, Thailand*
[2]*Parasitology Section, National Institute of Animal Health, Department of Livestock Development, Bangkhen, Bangkok 10900, Thailand*
[3]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand*
[4]*Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23, Bangkok 10110, Thailand*

Abstract

This study focused on the determination of the periodicity of microfilaria in naturally *Brugia malayi* infected adult cats and the evaluation of the efficacy of ivermectin as a microfilaricidal drug. Periodicity was achieved by collection of blood from 5 infected cats at 2-hour intervals over a period of 24 hours. The results showed that the parasites of all investigated cats were nocturnally subperiodic *B. malayi*, with the highest peak at approximately 24.00 hour. Five groups of cats were investigated for the efficacy of ivermectin. Groups 1 and 2 were given orally using two different single doses of ivermectin at 50 and 100 μg/kg body weight, respectively. Groups 3, 4 and 5 were subcutaneously injected using doses of 200, 400 and 1,000 μg/kg body weight, respectively. The results indicated that the microfilarial density of all ivermectin-treated cats had significantly dropped after 30 days of treatment ($p < 0.01$). In addition, similar results were observed when the same ivermectin dosages were given at day 30 after the first dose ($p < 0.05$). This study showed that the repeated doses of ivermectin given at 30-day interval were able to reduce the microfilaria of *B. malayi* in the blood circulation. This study also indicated that the oral and subcutaneous application of ivermectin was effective for rapid clearance of microfilaria in the blood circulation.

Keywords: *Brugia malayi*, ivermectin

Introduction

Human lymphatic filariasis is a major parasitic disease of the tropical and sub-tropical countries. Basically, elephantiasis is caused by the nematode parasite of *Brugia malayi*, which has been found in the southern part of Thailand [1-5]. In 1995, Phantana and his colleagues [6] had reported that approximately 4.3% of domestic cats in an endemic area of Narathiwat Province showed positive for microfilariae. This report corresponded to previous knowledge that wild animals such as the leaf-monkey (*Presbytis* spp) and domestic cats are extremely susceptible to subperiodic *B. malayi* infection [7-8]. These animal reservoirs play an important role in the re-emergence of *B. malayi* from time to time. This makes it difficult to eradicate elephantiasis in endemic areas.

Correspondence:
Kosum Chansiri
Tel: +66-2-6641000 ext 4605 Fax: +66-2-2600125
E-mail: kosum@psm.swu.ac.th

Over the past decade, several trials have indicated that single dose treatment with ivermectin was effective for the treatment of human lymphatic filariasis caused by *Wuchereria bancrofti* [9-12]. However, treatment with ivermectin of *B. malayi* in cats has not been reported elsewhere. This study demonstrated the periodicity of blood-circulating microfilariae and the efficacy of ivermectin as microfilaricidal drug for *B. malayi* in naturally infected cats.

Materials and methods

Animals

Thirteen adult male and female domestic cats from Narathiwat Province were investigated and diagnosed for the type of infection. Identification of *B. malayi* infection (17-321 microfilariae per 20 microliters of blood) was achieved by using morphological characteristics [13], different localization of acid phosphatase stained blood film [14] and PCR-based methods.

Assessment of microfilaremia

Five cats were determined for their microfilarial periodicity by collecting blood at 2-hour intervals for 24 hours. Twenty microliters of fresh blood were collected from the ear veins. Two duplicate lines of thick-blood samples were immediately made on a clean glass slide. The microfilariae were counted under a light microscope after Giemsa staining of blood films, microfilarial periodicity was further analyzed by the method of Aikat and Das [15].

Treatment protocol

Treatment of *B. malayi* infected cats was performed by five different experiments. The treatments of groups 1 and 2 were based on oral administration of ivermectin at dosages of 50 and 100 μg/kg body weight, respectively. The treatments of groups 3, 4 and 5 were conducted by subcutaneous injection of ivermectin at dosages of 200, 400 and 1,000 μg/kg body weight, respectively. Repeated treatment of each group was accomplished by using the same doses 30 days after the first dose. Blood samples were collected from the ear veins at 21.00 hour on day 0, day 30 and day 60. Blood-circulating microfilariae assessment was performed as described above.

Statistic analysis

Statistical evaluation of all treated cats before and after treatment was determined by Student's *t* test using the SPSS/PC+ program.

Infectivity index

The infectivity index indicated the probability of transmission that was related to the number of microfilariae in a certain amount of blood [16]. It was determined using the following equation:

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

"P" represents the probability of transmission; "m" represents the number of microfilariae per 2 ml blood.

$$\text{Infectivity index (\%)} = \frac{(Pn_1+Pn_2+\ldots\ldots) \times 100}{\text{No. of microfilariae per 2 μl blood}}$$

"n" represents the number of cases.

Results

Microfilarial periodicity

Periodicity was determined and it was found that the microfilariae were present in all of them in the peripheral blood at all times during the 24-hour period (Table 1). The mean value of blood microfilarial counts from each individual cat was represented as percentages of the peak count in 5 microfilaria-positive cats. The results showed that the microfilariae of all the cases were nocturnally subperiodic *B. malayi*. The parasites showed peak counts between 18.00-24.00 hours, with the highest peak at approximately 24.00 hours (Fig 1).

Efficacy of ivermectin for treatment of *B. malayi*

After the single oral and subcutaneous injection doses of ivermectin were administered, the rapid clearance of microfilaremia was achieved within 30 days (Table 2). The microfilarial density of all treatment groups had

Table 1 The average number (two duplicate blood films) of microfilariae of *B. malayi* and the percentages of the peak count (in parenthesis) in 5 microfilaria-positive cats from Narathiwat Province; blood taken at 2-hour intervals over a period of 24 hours.

Cat	Average number of microfilariae (% of the peak)											
No.	0600	0800	1000	1200	1400	1600	1800	2000	2200	2400	0200	0400
1	18.5 (29.1)	6 (9.4)	4 (6.3)	3.5 (5.5)	9.5 (14.9)	14.5 (22.8)	43.5 (68.5)	21 (33.1)	60.5 (95.3)	**63.5 (100)**	46 (72.4)	18 (28.3)
2	26 (34.9)	14.5 (19.5)	13.5 (18.1)	5 (6.7)	14 18.8)	27.5 (36.9)	28.5 (38.3)	62 (83.2)	62.5 (83.9)	**74.5 (100)**	52.5 (70.5)	21.5 (28.8)
3	30.5 (75.3)	37 (91.4)	5 (12.3)	20.5 (50.6)	21.5 (53.1)	14.5 (35.8)	29 (71.6)	33 (81.5)	34 (83.9)	**40.5 (100)**	23.5 (58.0)	17 (41.9)
4	14 (3.6)	23 (5.9)	39 (10.0)	88.5 (22.7)	99 (25.4)	206.5 (53.0)	99.5 (25.5)	146 (37.5)	**389.5 (100)**	155 (39.8)	102 (26.2)	36 (9.2)
5	16 (12.5)	46 (36.1)	98.5 (77.3)	107.5 (84.3)	87.5 (68.6)	103.5 (81.2)	**127.5 (100)**	58.5 (45.9)	39 (31.3)	59 46.3)	79 (61.9)	5.5 (4.3)
Mean	(31.1)	(32.5)	(24.8)	(33.9)	(36.2)	(45.9)	(60.8)	(56.2)	**(78.9)**	(77.2)	(57.8)	(22.5)

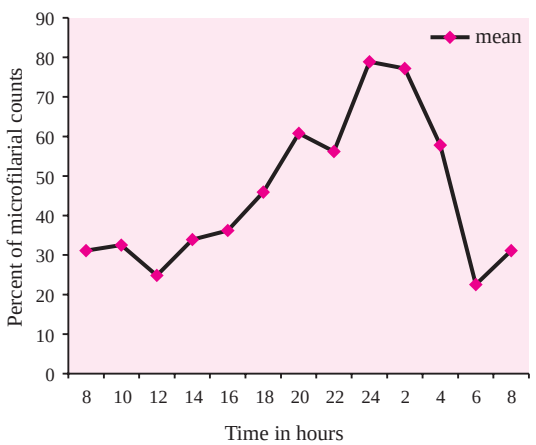

Fig 1 The periodicity of nocturnally sub-periodic *B. malayi* microfilariae from the cats in Narathiwat Province based on mean percent of microfilarial counts.

significantly dropped by 30 days after treatment with ivermectin at various doses ($p < 0.01$). However, microfilariae in the blood circulation was not totally eliminated within 30 days. Table 2 reveals that drug treatment by subcutaneous injection (groups 3, 4 and 5) was more effective than the oral drug treatment (groups 1 and 2). The second dosages of ivermectin were applied at day 30 and it was found that microfilaremia had decreased significantly at 30 days after the second treatment ($p < 0.05$). In spite of the repeated doses of ivermectin, the clearance of microfilaremia for all treatment groups was incomplete. In addition, the microfilaricidal effects were independent of the dosage of ivermectin, since the results of the recommended drug dose and higher doses were similar (200, 400 and 1,000 μg/kg body weight).

The infectivity index was also determined as an indication of the probability for transmission. It was found in Table 3 and Fig 2

Table 2 Blood circulating microfilarial density pre- and post-treatment for the 5 ivermectin dose groups. Differences were statistically significant ($p < 0.01$ after the first treatment and $p < 0.05$ after the second treatment).

Group	Doses (μg/kg BW)	Routes	Microfilarial density per 20 μl of whole blood (% reduction)		
			Pre-treatment	Post-treatment	
				30 days	60 days
1	50	oral	32	13 (59.3)	3 (76.9)
			140	43 (69.3)	24 (44.2)
2	100	oral	35	5 (85.7)	1 (80.0)
			160	83 (48.1)	32 (61.4)
3	200	subcutaneous	145	16 (88.9)	1 (93.7)
			52	13 (75.0)	3 (76.9)
			17	2 (88.2)	0 (100)
4	400	subcutaneous	20	34	nd
			53	1 (98.1)	1 (0)
			100	19 (81.0)	4 (78.9)
5	1,000	subcutaneous	32	7 (78.1)	1 (85.7)
			321	85 (73.5)	3 (96.5)
			50	4 (92.0)	nd
				$p < 0.01$	$p < 0.05$

nd = not detectable

that the ability of microfilarial transmission decreased when the repeated drug treatment was performed in all five groups of examined cats. The data showed that the infectivity indices of subcutaneous injection routes (groups 3, 4 and 5) were significantly different from those of the oral treatment modes (groups 1 and 2). Group 3, of which 200 μg ivermectin/kg body weight was used, demonstrated the highest efficacy of microfilarial clearance within 60 days after the repeated dosages at the 30-day interval. The increase of drug dosages, up to 400 and 1,000 μg/kg body weight, presented similar profiles of infectivity index.

Discussion

In this study, the results demonstrated that the microfilaremia of the naturally infected feline *B. malayi* was clearly a nocturnally subperiodic type; this was beneficial for diagnosis of the parasite at any time of the day. The results demonstrated that the repeated doses of ivermectin at different dosages (50-1,000 μg/kg body weight) given at a 30-day interval was able to reduce microfilaremia of *B. malayi*-infected cats. The dosage was sufficient for mass chemoprophylactic treatment in *B. malayi*-infected cats that remained potential transmission reservoirs. However, incomplete clearance of parasites was observed for all treatments. The results corresponded with those of Kumaraswami *et al* [10], where the ivermectin treatment of human bancroftian filariasis indicated the absence of dose dependence (25-200 μg/kg body weight).

According to the infectivity index profile, the efficacy of ivermectin treatment by subcutaneous injection was higher than that of

Table 3 Infectivity index of *B. malayi*-infected cats at pre-treatment (D0), post-treatment (D30 and D60) with ivermectin by oral and subcutaneous injection routes.

Group	n	Mean mf density (m)			Infectivity index (%)		
		D0	D30	D60	D0	D30	D60
1	2	8.6	2.8	1.3	100	93.9	73.0
2	2	8.8	4.4	1.6	100	98.8	80.8
3	3	7.1	1.3	0.13	99.9	64.4	12.3
4	3	5.8	1.8	0.17	99.7	83.5	16.7
5	3	13.4	3.2	0.13	100	95.9	12.3

oral treatment. Clearance of microfilariae was dramatically achieved after the second dose of subcutaneous injection with the same amount of ivermectin, whereas the oral treatments showed a slower effect. The data indicated that the possibility of transmission decreased when repeated dosages of treatments were performed.

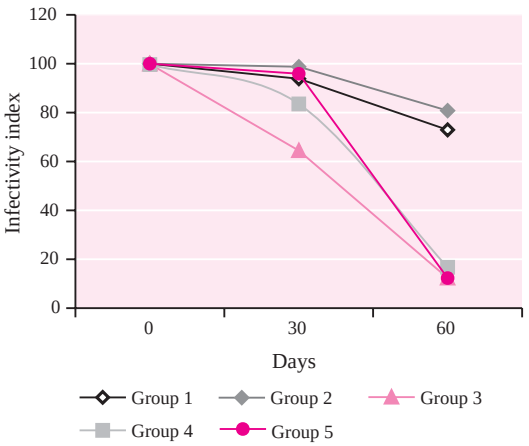

Fig 2 The average infectivity index profiles of per- and post-treatments of ivermectin by oral (groups 1 and 2) and subcutaneous injection (groups 3, 4 and 5) routes.

In addition, no adverse reactions to the ivermectin treatment were observed in the treated cats in this study, as previously reported by Oliva and Baldi [17] and Paradis *et al* [18].

In conclusion, a single dose of ivermectin was effective for the treatment of *B. malayi* microfilariae but not sufficient for complete clearance of microfilaremia. Hence, multiple doses of ivermectin at a 30-day interval are suggested for the prevention of disease transmission.

References

1. Iyengar MOT. Filariasis in Thailand. *Bull WHO* 1953;9:731-66.
2. Wongsathuaythong S, Punyagupta S, Sirivorasarn, P, Chularek P. Filariasis in Thailand: a preliminary report. *Royal Thai Army Med J* 1963;16:101.
3. Vasuvat C, Guptavanij P, Harinasuta C. Studies on the periodicity of microfilariae of *Brugia malayi* in Nakornsrithammaraj province. *J Med Assoc Thailand* 1966;49:16.
4. Harinasuta C, Sucharit S, Guptavanij P. Sub-periodic *Brugia malayi* in South Thailand. *Southeast Asian J Trop Med Public Health* 1970;1:162.
5. Guptavanij P, Harinasuta C, Sucharit S,

Vutikes S. Studies on sub-periodic *Brugia malayi* in southern Thailand. *Southeast Asian J Trop Med Public Health* 1971;2:44-50.

6. Phantana S, Sensathien S, Choompol S. Microfilaria in cat in Narathiwat southern Thailand. *Com Dis J* 1995;21:107-13.
7. Wharton RH. The biology of *Mansonia* mosquitoes in relation to the transmission of filaria in Malaya. *Institute for Medical Research Bulletin* 1962. No. 11, Kuala Lumpur.
8. Phantana S, Shutidamrong C, Chusattayanond W. *Brugia malayi* in a cat from southern Thailand. *Trans R Soc Trop Med Hyg* 1987;81:173-4.
9. Diallo S, Aziz MA, Ndir O, Badiane S, Bah IB, Gaye O. Dose ranging-study of ivermectin in treatment of filariasis due to *Wuchereria bancrofti*. *Lancet* 1987;1:1030.
10. Kumaraswami V, Ottesen EA, Vijayasekara V, Devi U, Swaminathan M, Aziz MA, *et al*. Ivermectin for the treatment of *Wuchereria bancrofti* filariasis. Efficacy and adverse reaction *JAMA* 1988;259:3150-3.
11. Cartel JL, Moulia-Pelat JP, Glaziou P, Nguyen LN, Chanteau S, Roux JF. Results of a safety trial on single-dose treatments with 400 mg/kg of ivermectin in bancroftian filariasis. *Trop Med Parasitol* 1992;43:263-6.
12. Kar SK, Patnaik S, Mania J, Kumaraswami V. Ivermectin in the treatment of bancroftian filarial infection in Orissa, India. *Southeast Asian J Trop Med Public Health* 1993;24:80-6.
13. Aoki Y, Nakajima Y, Katamina D. Studies on Malayan filariasis in Che-ju Is., Korea. 3. Microfilarial surface architecture of *Brugia malayi* (Che-ju strain) in comparison with that of *Brugia pahangi*. *Japanese J Trop Med Hyg* 1976;4:129-37.
14. Redington BG, Montgomery CA, Jervis HR, Hockmeyer WT. Histochemical differentiation of the microfilariae of *Brugia pahangi* and sub-periodic *Brugia malayi*. *Ann Trop Med Parasitol* 1975;69:489-92.
15. Aikat TK, Das M. A modified statistical method for analysis of periodicity of microfilaria.World Health Organization, WHO/FIL/76.142, 1976. Geneva.
16. Manabu S. Human filariasis: a global survey of epidemiology and control. Tokyo: University of Tokyo Press; 1976. p.700-5.
17. Oliva G, Baldi L. Trial of ivermectin for the treatment of endo and ectoparasites of the cat. *Acta Med Vet* 1988;34:471-7.
18. Paradis M, Scott D, Villeneuve A. Efficacy of ivermectin against *Cheyletiella blakei* infestation in cats. *J Am Anim Hosp Assoc* 1990;26:125-8.