長崎大学学術研究成果リポジトリ

NAOSITE

Nagasaki university's Academic Output SITE

Title	Acanthocheilonema viteae in Mastomys coucha : Combination Effect of An Immunostimulator (CDRI compound 86/448) and Antifilarial agents on Establishment of Infection
Author(s)	Bhatnagar, Sunita; Fatma, Nigar; Chatterjee, R.K.
Citation	Tropical medicine 37(4). p151-157, 1996
Issue Date	1996-03-29
URL	http://hdl.handle.net/10069/4715
Right	

151

Acanthocheilonema viteae in *Mastomys coucha:*

Combination Effect of An Immunostimulator (CDRI compound 86/448) and Antifilarial agents on Establishment of Infection

Sunita Bhatnagar, Nigar Fatma and R.K. Chatterjee

Division of Parasitology, Central Drug Research Institute
Lucknow 226 001, India

Abstract: Effect of two antifilarials ivermectin (microfilaricidal) and CDRI Comp. 82/437 (macrofilaricidal) in combination with immunostimulator (CDRI Comp. 86/448) was evaluated on establishment of *Acanthocheilonema viteae* infection in *Mastomys coucha*. The immunostimulator along with the antifilarials was administered on single occasion (Day 0 of larval exposure). Immunostimulator when given in combination with macrofilaricidal agent (82/437), revealed significantly less percentage of worm recovery over untreated control as well as over treated (with either immunostimulator or antifilarial alone) infected controls. It is, thus surmised that establishment of filarial infection is affected by immunostimulant along with antifilarial agent.

Key words: Acanthocheilonema viteae, Mastomys coucha, Immunostimulator, Compound 82/437, Microfilaricidal, Parasite establishment, Macrofilaricidal, Compound 86/448.

INTRODUCTION

Filariasis is a major parasitic disease of great public health importance affecting millions of people in India alone (NICD, 1994). Apart from chemotherapeutic measures, control of the disease in endemic areas largely depends upon the efficient preventive measures such as protection from infective larval invasion from vector bites. It is now well documented that like many other parasitic infections, filarial parasites also cause immunosuppression (Portaro *et al.,* 1976; Ottesen *et al.,* 1977; Piessens *et al.,* 1980, a, b) which help them in their establishment in the host. Stimulation of host's immune responsiveness by use of immunomodulators thus appears to be a logical approach in counteracting establishment process of infective larvae. This has been amply substantiated from our earlier studies in which establishment of *A. viteae* and *B. malayi* infections has been shown to be jeopardized significantly when host was primed with immunomodulators before infective exposure (Misra *et al.,* 1991; Murthy *et al.,* 1992). On the other hand there are certain antifilarial agents which are known to act only partially on developing forms of filariae. Combination therapy with such antifilarial agents along with immunopotentiator appears to be a practical proposition in crusade against establishment of invading and developing larvae in the host.

Previous studies in our laboratory have indicated that chemotherapeutic efficacy of antifilarial agent can also be enhanced by using it along with immunomodulators (Chatterjee *et al.*, 1988; Fatma *et al.*, 1994). Thus, application of combination of immunostimulator and antifilarial agent might exert better and more efficient protection against infection. In the present study, efforts have been made to prevent the establishment of *Acanthocheilonema viteae* in *Mastomys coucha* with combination therapy using CDRI compound 82/437 or ivermectin along with a synthetic immunostimulator, CDRI Compound 86/448.

MATERIALS AND METHODS

Experimental model: *Acanthocheilonema viteae* in *Mastomys coucha* was used as experimental model. Six to eight weeks old male mastomys were exposed subcutaneously with 50 infective larvae (L3) of *A. viteae* collected from freshly dissected infected ticks (*Ornithodoros moubata*) following broadly the technique of Singh *et al.* (1988).

Immunomodulator: CDRI Compound 86/448, a synthetic glycopeptide, was used in the present study as immunostimulator. The Compound 86/448 is a structurally related congenor of muramyl dipeptide (MDP) and was originally synthesized in this Institute (Haq *et al.*, 1990).

Antifilarials: Two antifilarials used in the study were ivermectin and a synthetic agent, compound 82/437. Ivermectin was procured from Merck Sharpe and Dohme, New Jersey. The compound 82/437 was originally synthesized in this Institute (Abuzar *et al.*, 1986). The agent is a benzimidazole derivative (2,2' Dicarbomethoxy amino 5−5' dibenzimidazolyl ketone) having macrofilaricidal activity against three filarial species *L. carinii, A. viteae* and *B. malayi* in rodents (Fatma *et al.*, 1989).

Drug preparation and Dose schedule: Compound 86/448 was prepared in sterile distilled water and used at a dose of $250 \mu g$/animal on single occasion subcutaneously. Ivermectin and compound 82/437 were used at $15 \mu g/kg \times 1$ and $100 mg/kg \times 1$ orally respectively. Both the compounds were prepared in distilled water containing 0.1% Tween−80. Dose of antifilarials and immunomodulator corresponded to the ED_{50} values.

Experiment: Four experimental groups, each consisting of 8−10 animals were used. Different groups receiving treatment and controls were:

1) treated with antifilarials only (ivermectin/Comp. 82/437).
2) treated with immunomodulator only (86/448).
3) treated simultaneously with antifilarial and immunomodulator.
4) untreated controls.

Animals belonging to different experimental groups were exposed to 50 L3 of *A. viteae* 2 hrs after administration of drug and/or immunomodulator. Untreated animals were also exposed under identical conditions. The effect of individual or combination of drugs was assessed by observing recovery of adult parasites on day 60 i. e., at the start of patency of infection.

Adult worm recovery: On day 60 of infective exposure, adult parasites were recovered from subcutaneous tissues of the infected animals. The percentage reduction in worm recovery was calculated by comparing the number of live adult worms recovered from experimental animals

with that recovered from untreated infected controls.

Statistical analysis: The data was analysed and p values were taken out using Student's T test.

<div align="center">RESULTS</div>

Effect of Compounds 86/448 and ivermectin: Table 1 shows the effect of 86/448, ivermectin and their combination on establishment of *A. viteae* infection in mastomys. Treatment with compound 86/448 and ivermectin resulted in 60.2% reduction in adult worm recovery over control ($p < 0.01$). Ivermectin and compound 86/448 individually exerted respectively 52.8% and 42.9% reduction in worm recovery over untreated infected control.

Table 1. Effect of ivermectin, compound 82/437 and their combination with immunomodulator compound 86/448 on establishment of *A. viteae* infection in *M. coucha*

Treatment	Dose*	No. of animals (no. of expts.)**	No. of worms recovered Mean±SD	% establishment of worms	% reduction in worm recovery	% reduction in worm recovery over control
Ivermectin (I)	15μg/kg×1	10 (3)	13.4±4.6	26.8±9.3	73.2	52.8
82/437 (II)	100mg/kg×1	12 (3)	18.5±5.4	37.0±10.9	63.0	34.8
86/448 (III)	250μg/animal×1	12 (3)	16.2±4.4	32.4±8.8	67.6	42.9
Ivermectin (IV) 88/448	15μg/kg×1 + 250μg/animal×1	10 (3)	11.3±1.5	22.6±3.0	77.4	60.2 (P<0.01)
82/437 (V) 86/448	100mg/kg×1 + 250μg/animal×1	12 (3)	4.3±3.8	8.7±7.8	91.3	84.8 (P<0.001)
Infected control	Vehicle	10 (3)	28.4±4.2	56.8±8.4	43.2	0.0

* Comp. 86/448 was administered subcutaneously whereas other agents were fed orally
**All animals were exposed to 50 L3 of *A. viteae*

<div align="center">

Table 1a. Statistical significance of percent reduction in worm recovery
(over control) amongst treated groups.

Treated groups	P values
I vs II	p<0.2
I vs III	p<0.5
I vs IV	p<0.1
I vs V	p<0.005
II vs III	p<0.5
II vs IV	p<0.001
II vs V	p<0.001
III vs IV	p<0.02
III vs V	p<0.001
IV vs V	p<0.001

</div>

I = Ivermectin
II = 82/437
III = 86/448
IV = Ivermectin+86/448
V = 82/437+86/448

Effect of compounds 86/448 and 82/437: A significant reduction (84.8%) in establishment of *A. viteae* infection was observed when animals were exposed following treatment with combination of compounds 86/448 and 82/437. Compound 82/437 alone exerted 34.8% reduction in adult worm recovery over controls (Table 1). Statistical significance of percent reduction in worm recovery (over control) amongst treated groups is shown in Table 1a.

<div align="center">DISCUSSION</div>

Control of fillariasis appears difficult in absence of any effective marcrofilaricide. Nevertheless, continuous infective larval exposure through vectors in endemic area challenges our strategies in chemotherapy. One of the major logistics for control of filariasis would be the protection of the population from establishment in host. This can be achieved either by vector control or by drugs having lethal effect on developing forms of filarial larvae in the host. The latter strategy i. e. control by use of filarial larvicidal agents has been evaluated in the present investigation. Earlier, a number of attempts on prophylaxis by chemical agents have been made using single or combination of antifilarial agents against different experimental filarial infections (Denham and Brandte, 1980; Zahner *et al.,* 1987; McCall *et al.,* 1993). Most of these studies however, did not yield desired results. The existing drug diethylcarbamazine (DEC) has strong effect on microfilariae but its effect on other developing forms of target parasite, *W. bancrofti* is not convincing (Jordan, 1958). However, the drug has been shown to be larvicidal with multiple doses as revealed in several experimental studies (Denham *et al.,* 1978; Mak and Lim, 1983) including its use as chemoprophylactic agent against loaisis (Duke 1963).

As immune system plays an important role in exertion of drug activity in host (Ottesen *et. al.,* 1977; Kwa and Mak, 1980; Piessens *et al.,* 1980) the strategy taken up in the present study is to boost the antifilarial larvicidal activity potential of the drug with the help of an immunostimulator. Two candidate antifilarials CDRI compound 82/437 and ivermectin which have been shown earlier to exert moderate action against infective larvae (Fatma *et al.,* 1989; Singh *et al.,* 1990) have been used in the present investigation along with immunostimulator to potentiate their larvicidal property. It may be recalled that certain immunostimulators themselves can effect to an extent the growth, development and establishment of larvae in the host (Misra *et al.,* 1991; Murthy *et al.,* 1992).

The single dose therapy with any individual antifilarial agent led to 35 to 53% effect on establishment of adult worms when compared with controls. The maximum efficacy of 53% was demonstrated with ivermectin alone followed by immunostimulator compound 86/448 with 42.9% activity and an activity of 34.8% was displayed by the adulticidal agent, compound 82/437. It is interesting to observe that though no significant enhancement in activity against worm establishment occurred with combination of ivermectin and the immunostimulator, very significant improvement in action of 82/437 occurred when this antifilarial was administered along with immunostimulator. The better performance of combination of 82/437 and immunostimulator (86/448) was primarily due to complementary action of the two agents against establishment of filariids. In our earlier studies (Misra *et. al.,* 1991) it was shown that peptide immunostimulators

like MDP and its derivatives could suppress filarial establishment in rodent hosts and subsequent development of microfilaraemia through enhancement in activity of reticuloendothelial system including macrophage functioning. *A. viteae* used in the present study is known to be susceptible to reactive oxygen intermediates (Batra *et al.*, 1990). Nevertheless, the parasite also possesses an active enzyme system like SOD and catalase to protect itself against host's oxidant attack. Compound 82/437 inhibits host's oxidant attack. Compound 82/437 also inhibits significantly the antioxidants (catalase and glutathione peroxidase) of parasite rendering them prone to H_2O_2 toxicity leading to death (Batra *et al.*, 1992). Thus on one hand presently used peptide immunostimulator enhanced the release of reactive oxygen intermediates and on the other hand compound 82/437 disrupted the antioxidant defence system of the parasites leading to chain of activity against establishment of filarial larvae in the host.

Though the immunostimulator used as combinant with ivermectin was the same, the mechanism of exertion of activity of the two agents differ (Turner and Schaeffer, 1989; Mak *et al.*, 1991; Pal *et al.*, 1991). None of these thus benefitted from the activity of the other with the result that no improvement in activity of the combination was observed. The combination of compound 82/437 and the peptide immunostimulator should be investigated further for their efficacy against establishment of filarial infection in higher models eg. monkey with human filarial infection.

REFERENCES

1) Abuzar, S., Sharma, S., Fatma, N., Gupta, S., Murthy, P.K., Katiyar, J.C., Chatterjee, R.K. & Sen, A.B. (1986): Studies on potential filaricides Part 18: Synthesis of 2,2' dicarbomethoxyl amino 5−5' dibenzimidazolyl ketone and related compounds as potential anthelmintics. J. Med. Chem., 29, 1296−1299.

2) Batra, S., Chatterjee, R.K. & Srivastava, V.M.L., (1990): Antioxidant enzymes in *Acanthocheilonema viteae* and effect of antifilarial agents. Biochem. Pharmacol., 40, 2363−2369.

3) Batra, S., Chatterjee, R.K. & Srivastava, V.M.L. (1992): Antioxidant enzyme system in *Litomosoides carinii* and *Setaria cervi* : Effect of a macrofilaricidal agent. Vet. Parasitol., 43, 93−103.

4) Chatterjee, R.K., Fatma, N., Jain, R.K., Gupta, C.M. & Anand, N. (1988): *Litomosoides carinii* in rodents: Immunomodulators in potentiating action of diethylcarbamazine. Jpn. J. Exp. Med., 58, 243−248.

5) Denham, D.A. & Brandte, E. (1980): Chemoprophylactic activity of flubendazole against adult *Brugia pahangi* transplanted into the peritoneal cavities of jirds. J. Parasitol., 66, 933−934.

6) Denham, D.A., Suswillo, R.R., Rogers, R. & McGreevy, P.B. (1978): Studies with *Brugia pahangi* 17: The anthelmintic effects of diethylcarbamazine. J. Parasitol., 64, 463−468.

7) Duke, B.O.L. (1963): Studies on the chemoprophylaxis of Loaisis II Observations on diethylcarbamazine

citrate (Banocide) as a prophylactic in man. Ann. Trop. Med. Parasitol., 57, 82—96.

8) Fatma, N., Mathur, K.B. & Chatterjee, R.K. (1994): Chemotherapy of experimental filariasis: enhancement of activity profile of ivermectin with immunomodulators. Acta Tropica, 57, 55—67.

9) Fatma, N., Sharma, S. & Chatterjee, R.K. (1989): 2,2' Dicarbomethoxylamino 5,5' dibenzimidazolyl ketone a new antifilarial agent. Acta Tropica, 46, 311—321.

10) Haq, W., Puri, A., Kundu, B., Saxena, R.P., Kapil, A., Mathur, K.B. & Saxena, K.C. (1990): A process for the synthesis of N-glycyl, N (L-abanyl-D-isoglutaminyl)-L-Cysyl-N-alkylamides possessing high immunostimulant activity. Patent application filed (434/DEL/90).

11) Jordan, P. (1958): Action of DEC *in vitro* on infective larvae of *W. bancrofti.* Brit. J. Pharmacol. Chemother., 13, 318—320.

12) Kwa, B.H. & Mak, J.W. (1980): Specific depression of cellular-mediated immunity in Malayan filariasis. Trans. R. Soc. Top. Med. Hyg., 74, 522.

13) Mak, J.W. & Lim, P.K.C. (1983): Studies on the chemoprophylaxis of subperiodic *Brugia malayi* infection in the leaf monkey (*Preshytis melalophos*) with diethylcarbamazine citrate. Z. Parasitenk., 69, 677—680.

14) Mak, J.W., Navaratnam, V. & Ramachandran, C.P. (1991): Experimental Chemotherapy of lymphatic filariasis. A review. Ann. Trop. Med. Parasitol., 85, 131—137.

15) McCall J.W., Coleman, S.U., Jones K.L., Petit, T.A. & Klei T.R. (1993): Prophylactic activity of tetracycline against *B. pahangi* infection in jirds (*Meriones unguiculatus*). J. Parasitol., 79, 775—776.

16) Misra, S., Singh, D.P., Gupta, C.M., Chatterjee, R.K. & Anand, N. (1991): *Acanthocheilonema viteae* in *Mastomys natalensis* : Effect of immunomodulator on establishment and course of infection. Med. Sci. Res., 19, 53—55.

17) Murthy, P.K., Haq, W., Tyagi, K., Mathur, K.B. & Chatterjee, R.K. (1992): Effect of immunostimulator on establishment and course of infection. Trop. Med., 34, 63—69.

18) NICD (1994): Proceedings of National workshop to formulate strategy for *Brugia malayi* eradication from India. 15 & 16th December NICD, Karaparamba, Calicut, Kerala, India.

19) Ottesen, E.A., Walter, P.F. & Heck, L. (1977): Specific cellular unresponsiveness in human filariasis. Immunol., 33, 413—421.

20) Pal, R., Anuradha, Rizvi, S.Y., Kundu, B., Mathur, K.B. & Katiyar, J.C. (1991): *Leishmania donovani* in hamster: Stimulation of non specific resistance by some novel glycopeptide and impact on therapeutic efficacy. Experientia, 47, 4010—4016.

21) Piessens, W.F., McGreevy, P.B., Piessens, P.W., McCreevy, M., Koiman, I., Saroso, J.S. & Dennis, D.T. (1980a): Immune response in human infections with *Brugia malayi* specific cellular responsiveness to filarial antigens. J. Clin. Invest., 65, 172—179.

22) Piessens, W.F., McGreevy, P.B., Ratiwayanto, S., McGreevy, M., Piessens, P.W., Koiman, I., Saroso, J.S. & Dennis, D.T. (1980b). Immune response in human infections with *Brugia malayi* correlation of cellular and humoral reactions to microfilarial antigens with clinical status. Am. J. Trop. Med. Hyg., 29, 563—570.

23) Portaro, J.K., Britton, S. & Ash, L.R. (1976): *Brugia pahangi* : depressed mitogen reactivity in filarial infections in the jirds (*Meriones unguiculatus*). Expt. Parasitol., 40, 438—446.

24) Singh, D.P., Misra, S., & Chatterjee, R.K. (1988): Response of *Dipetalonema viteae* in *Mastomys natalensis* to known antifilarials. Ind. J. Exp. Biol., 26, 48—52.

25) Singh, D.P., Misra, S. & Chatterjee, R.K. (1990): A new technique in *in vitro* assay of antifilarials using different life forms of *Acanthocheilonema viteae.* Jap. J. Exp. Med., 60, 303—309.

26) Turner, M.J. & Schaeffer, J.M. (1989): Eds. Campbell W.C. Mode of action of ivermectin. In. Ivermectin

& Abamectins Springer Verlag, New York, 73−88.

27) Zahner, H., Striebel, H.P., Schultheiss, K., Schutze H.R. (1987): Chemoprophylactic activity of some benzazole derivatives in experimental filarial infections. Trop. Med. Parasitol., 38, 259.