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Brugia malayi in *Mastomys natalensis:* efficacy of mebendazole in combination with Freund's complete adjuvant

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Key words: *Brugia malayi,* Freund's adjuvant, *Mastomys,* mebendazol

Abstract. The chemotherapeutic efficacy of mebendazole given in combination with Freund's complete adjuvant (FCA) against *Brugia malayi* in multimammate rat was evaluated. Animals treated with mebendazole, orally at 200 mg/kg × 5 consecutive days along with FCA given subcutaneously (s.c.) on day −10, day 0 and day +15 of the drug treatment killed 48.51 % of the adult worms. This drug given alone at the same regimen and by the same route showed only 18.7 % mortality rate on adults. Mebendazole given intraperitoneally along with FCA given s.c., however, was four times more efficacious as filaricide than mebendazole alone. Nevertheless, the animals receiving FCA alone also revealed 23.5 % mortality rate of adult worms. The animals receiving a combination therapy or FCA alone showed significant increase in antibody titre to the filariae which however decreased in the later stages. No enhancement of antibody level could be detected in animals treated with mebendazole alone. The non-specific immunopotentiation induced by FCA appeared to play a major role in enhancing the activity of mebendazole.

Participation of body's defence mechanism is vital for effective chemotherapy of infections. The therapeutic efficacy of many drugs, indicated for various bacterial and viral infections and even cancer, can be enhanced by immuno-potentiators (Mathe et al. 1969, Adlan et al. 1972, Kreska 1979, Drews 1980). The use of immunopotentiators specially in some parasitic diseases might have special significance as these infections induce immunosuppression (Ottesen et al. 1977, Waller 1978, Piessens et al. 1980a, b, 1982) which may lead to poor drug action. Recently the action of an antileishmanial drug was shown to be enhanced when applied along with an immunopotentiator (Adinolfi and Bonventre 1985). However, no serious effort has been made so far to enhance activity of drugs with the help of immunomodulators. Mebendazole, a broad spectrum anthelmintic (Vanden Bossche 1981), shows unpredictable activity against filarial parasites when administered by oral route. Although the principal reason for the poor antifilarial action of this drug is its low absorption through gastrointestinal tract (Denham et al. 1978), it is contemplated that whatever quantum of drug (mebendazole) is absorbed, it could possibly be made more effective by stimulating the immune status of the host. With this objective the present study was conducted to evaluate antifilarial efficacy of mebendazole along with Freund's complete adjuvant (FCA) against experimental filarial infection.

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MATERIALS AND METHODS

Experimental model: Subperiodic strain of human filaria, *Brugia malayi* was maitained experimentally in *Mastomys natalensis* through the vector, *Aedes aegypti* (Murthy et al. 1983). Only the animals showing progressive rise in microfilaraemia were included in the study.

Immunomodulator: Freund's complete adjuvant (DIFCO Laboratories, Michigan) was used as immune promoter.

Dose-schedule of drugs: Mebendazole (Unichem Laboratories, Bombay) was suspended in 1 % Tween 80 in distilled water and administered orally at 100 and 200 mg/kg or 6.25, 12.5, 25.0 and 50.0 mg/kg body weight intraperitoneally for 5 consecutive days.
FCA was administered at 0.1 ml/animal subcutaneously on days −10, 0 and +15 of start of mebendazole treatment.
Infected animals receiving 1 % Tween 80, FCA or mebendazole alone served as controls.
At least 5–6 animals in two sets of experiments were used in each dose group.

Assessment of microfilaricidal activity: Microfilaraemia was recorded on day 0 and day 8 and thereafter at weekly interval till day 91 of start of treatment by examining 20 mm^3 of tail blood. Alterations in microfilaraemia at different time intervals in relation to day 0 microfilarial (mf) count of each animal was determined and expressed as percent change in mf count (Tyagi et al. 1986).

Assessment of macrofilaricidal activity: All the animals were sacrificed on day 91 of start of treatment and the condition of adult parasites recovered from different organs of the body was examined thoroughly (Tyagi et al. 1986) for viability, cell adhesion on body surface and reproductive status of female worms. Percent mortality of adult worms was calculated following the methods of Lammler et al. (1978) and Misra et al. (1981).

Assessment of immune response: The filaria specific antibody response of animals was measured using indirect haemagglutination test (IHA), following the technique of Boyden (1951) with minor modifications to suit the present conditions. The titres were monitored on days −11, +28, +56 and +91 of drug treatment and the values were expressed as $\log_2$ titres.
Statistical analysis: Data were analysed by student *t*-test and/or Mann Whitney 'U' test.

RESULTS

Effect on microfilaraemia: The microfilaricidal action of mebendazole alone or in combination with FCA is shown in Table 1 and Fig. 1. The drug alone at 200 mg/kg (oral) did not exert any microfilaricidal action against *B. malayi*. However, when it was administered along with FCA, there was 28.69 % reduction in mf count by day 8 and microfilaraemia remained suppressed (−69 %) till the day of sacrifice (i.e. day 91). Treatment with lower dose of mebendazole (100 mg/kg) along with FCA had no effect on microfilaraemia till day 91.

Intraperitoneal administration of mebendazole alone at 25 mg/kg produced gradual fall in microfilarial count. However, at 50 mg/kg dose level, all the treated animals became amicrofilaraemic by day 35 (Table 1, Fig. 2).

In combination with FCA, mebendazole caused significant fall in microfilaraemia even at 6.25 mg/kg by intraperitoneal route. The combination effect was further improved when the dose of the drug was increased to 12.5 mg/kg (Table 1).

Untreated infected animals showed progressive rise in mf count. However,

Table 1. Microfilaricidal action of mebendazole along with FCA against *B. malayi* in *Mastomys natalensis*

Route of application	Dose (mg/kg × 5 days) of mebendazole	No. of animals at		Initial count[+] mean ± SD	% change in microfilarial count** on day			
		start	end		8	35	63	91
Oral	100	5	4	44.75 ±15.39	6.23 ±84.57	72.15 ±133.68	12.18 ±73.67	46.88 ±173.14
	200	5	3	36.0 ±26.96	6.55 ±48.30	37.05 ±38.66	132.15 ±162.44	196.53 ±222.80
	100 + FCA*	6	6	93.83 ±133.69	59.18 ±162.95	92.39 ±158.80	46.20 ±53.08	69.39 ±28.79
	200 + FCA*	6	6	144.33 ±51.77	−28.69 ±44.29	−18.34 ±102.32	−62.69 ±27.54	−69.72 ±28.02
Intra-peritoneal	12.5	5	4	43.00 ±6.33	19.99 ±56.54	33.45 ±41.97	70.08 ±146.27	55.60 ±109.42
	25.0	5	5	32.00 ±8.43	34.24 ±58.72	−64.28 ±27.08	−87.76 ±9.03	−92.72 ±4.85
	50.0	5	5	33.80 ±13.39	56.79 ±56.38	−100	−100	−100
	6.25 + FCA*	5	3	68.33 ±31.21	7.90 ±23.72	−85.20 ±5.42	−41.43 ±30.82	−32.27 ±40.94
	12.5 + FCA*	5	5	67.00 ±36.28	−46.79 ±18.44	−99.76 ±0.53	−100	−100
	FCA*	6	5	106.17 ±67.96	−7.94 ±65.03	20.06 ±62.29	14.08 ±49.70	−16.57 ±73.36
	Untreated	10	10	43.80 ±30.30	45.81 ±94.15	116.31 ±130.72	160.57 ±154.01	369.94 ±262.00

* – FCA by subcutaneous route
** – Mean ± S. D.
[+] – Initial microfilarial count represent 100 %

animals receiving FCA only also showed supression in microfilaraemia (Table 1) which was significant when compared with untreated control ($P < 0.01$).

Effect on adult worms: Table 2 depicts the results of macrofilaricidal action of mebendazole by oral and i.p. routes with or without FCA. Oral administration of mebendazole alone for 5 consecutive days showed weak adulticidal effect at 100 mg (10.25 % death) or 200 mg/kg (18.70 % death) dose levels. In combination with FCA, the drug exhibited 28.73 and 48.51 % adulticidal actions, respectively at 100 and 200 mg/kg orally which were however not statistically significant when compared with only FCA treated animals. Nevertheless 60 and 90 % of females recovered from animals treated with the drug respectively at 100 and 200 mg/kg (oral) along with FCA contained distorted ova/mf in the uteri.

By intraperitoneal route mebendazole brought about 6.51 and 96.75 % death of adult parasites respectively at 12.5 and 25 mg/kg × 5 days. The activity was further enhanced when given in combination with FCA (32.25 and 100 % death of adults

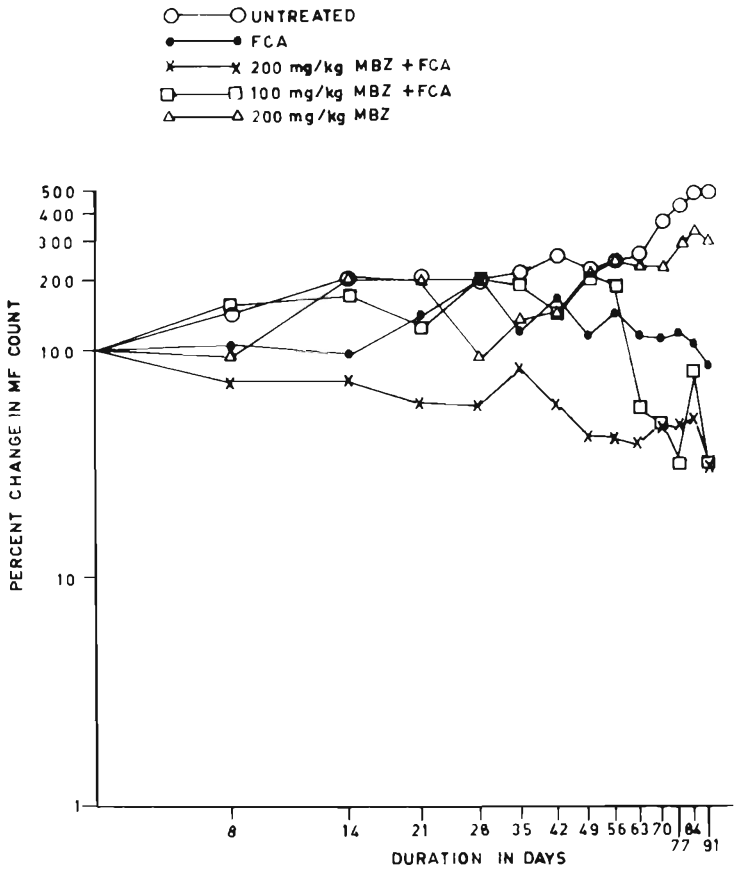

Fig. 1. Microfilaricidal action of combination of mebendazole (oral) and FCA.

Table 2. Macrofilaricidal action of mebendazole along with FCA against *B. malayi* in *Mastomys natalensis*

Route of application	Dose mg/kg	Percent mortality (Mean ± SD)		
		Male	Female	Total
Oral	100	15.00 ±52.60	7.58 ±25.93	10.57 ±23.93
Oral	200	20.00 ±34.64	17.81 ±36.25	18.70 ±35.44
Oral	100 + FCA*	36.67 ±46.33	20.09 ±52.99	28.73 ±48.57
Oral	200 + FCA*	50.00 ±54.77	47.49 ±46.99	48.51 ±46.47
Intraperitoneal	12.5	5.00 ±50.00	7.54 ±40.90	6.51 ±43.28
Intraperitoneal	25.0	100.00	94.52 ±12.25	96.75 ±7.27
Intraperitoneal	50.0	100.00	100.00	100.00
Intraperitoneal	6.25 + FCA*	40.00 ±20.00	26.94 ±20.93	32.25 ±12.42
Intraperitoneal	12.5 + FCA*	100.00	100.00	100.00
Intraperitoneal	FCA*	12.00 ±46.04	32.48 ±22.74	23.58 ±29.65

* - FCA by subcutaneous route

at 6.25 and 12.50 mg/kg of the drug, respectively). However, animals receiving FCA only also exerted 23.58 % macrofilaricidal action and 60 % of the remaining live females were sterile. Adult worms recovered from untreated animals were healthy with females having normal uterine contents.

Antibody response: Antibody response of animals receiving mebendazole (200 mg/kg, orally) alone or with FCA and FCA alone is shown in Fig. 3. The filaria specific antibody level in animals treated with mebendazole plus FCA or FCA alone showed significant increase on day 56, the titre being respectively 10 and 11 ($P < 0.01$) over controls (titre: 7). However, a decrease in titre was recorded in animals of both groups on day 91. In animals with only mebendazole treatment, antibody titre remained more or less at pretreatment level till day 91 of observation period.

DISCUSSION

In spite of excellent filaricidal action by parenteral route which is however not recommended due to severe local reactions, mebendazole could not be developed as an antifilarial drug because of its highly unpredictable effect on filariids by oral

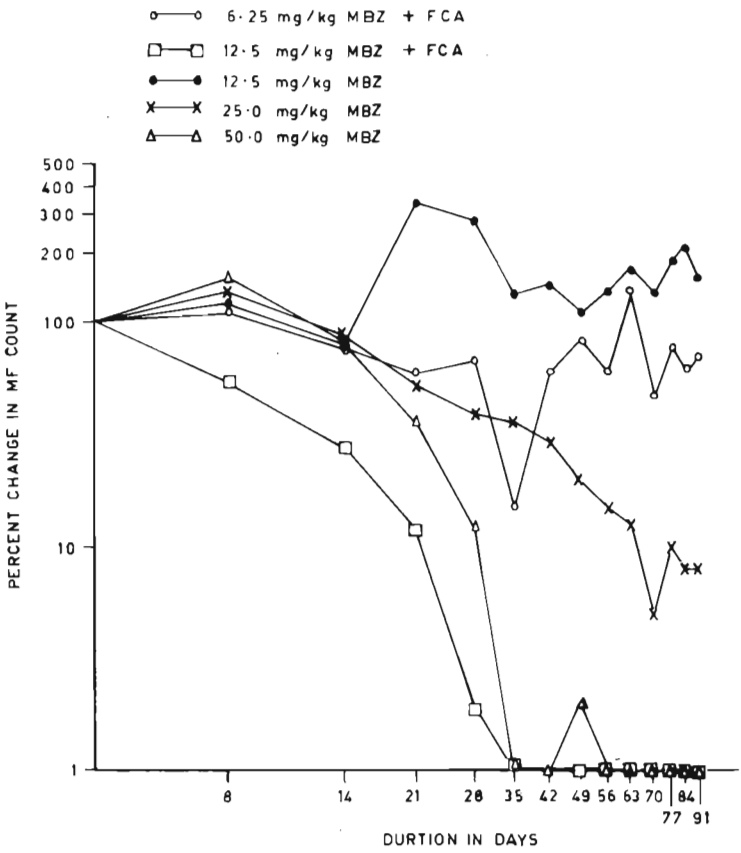

Fig. 2. Microfilaricidal action of combination of mebendazole (intraperitoneal) and FCA.

route (Denham et al. 1978). Nevertheless, later study indicated that oral efficacy of mebendazole could be improved when given along with levamisole, an antiparasitic agent with immunomodulatory property (Bernberg et al. 1979) against *Acanthocheilonema perstans* in human subjects. This fact opened up the possibility that activity of this antifilarial agent could be enhanced or synergised with immunoadjuvants.

In the present study, macrofilaricidal action of mebendazole was increased significantly even by oral route when given in combination with FCA. Though the exact mechanism of enhancement of activity of such combination is not clear, it is expected to be through promotion of activity of immune system of treated host. Similar observations were made by Adinolfi and Bonventre (1985) and George et al. (1986) where they found that antileishmanial and antiviral activities were enhanced when administered along with synthetic peptides. Under the present conditions, the immunoadjuvant (FCA) improved the activity of mebendazole whether given orally or intraperitoneally. However, enhancement of activity

was more perceptible with the later route of administration of the drug. Thus 100 % adulticidal effect was observed with 12.5 mg/kg + FCA by intraperitoneal route which was one quarter of the effective dose of mebendazole alone. By oral route, FCA enhanced adulticidal activity of the drug which was about 2.5 times more than the effect with the drug alone.

It was difficult in the present study to correlate between microfilaricidal and adulticidal actions following treatment with the drug alone or its combination with FCA. Thus though there was certain percentage of death of adult parasites (10.5 and 18.70 %, respectively, with 100 and 200 mg/kg of mebendazole by oral route), there was no reduction in the level of microfilaraemia up to day 91 of observation period. It is also apparent that fall in mf level occurs only when percent mortality of adult worms exceeds 20 % (Tables 1 and 2).

It is interesting to note that FCA alone also caused certain percentage of mortality (23.58 %) of adult worms possibly by activating macrophages which stimulated antibody production in T-dependent antibody producing cells (Roitt 1980). Bomford (1980) also observed immuno-potentiating effect of FCA in rodents. In may be recalled that immunosuppression occurs in host during the course of filarial infection (Portaro et al. 1976, Ottesen et al. 1977, Grove and Forbes 1979, Mehta et al. 1980 and Piessens et al. 1980 a, b).

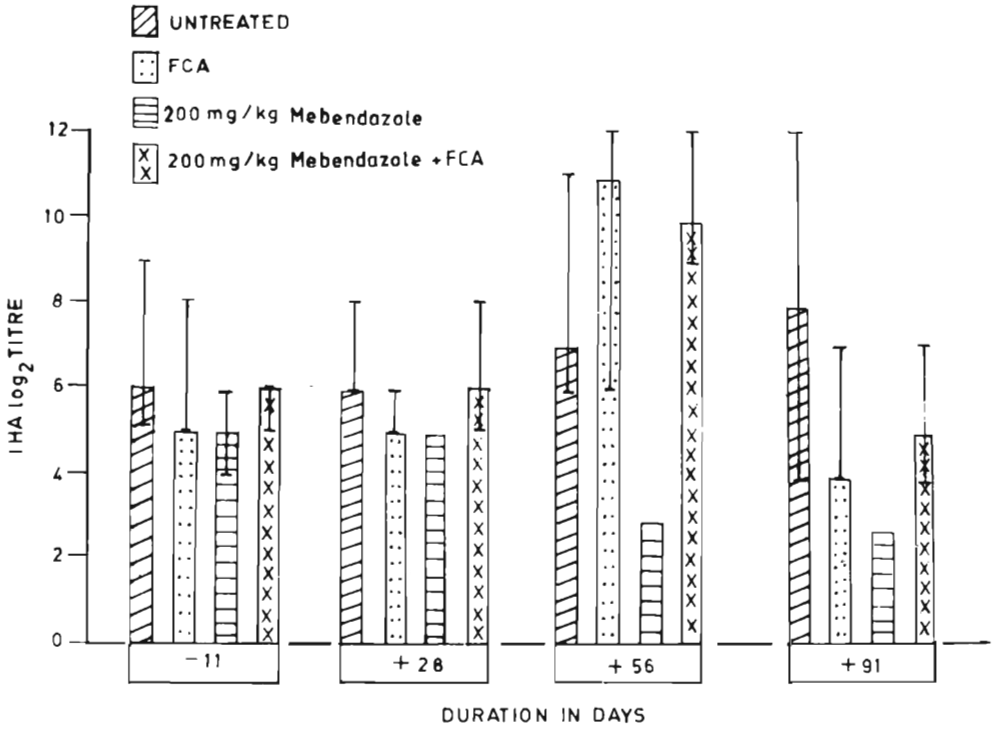

Fig. 3. Antibody response of treated animals (median with range).

Administration of FCA possibly led to reversal of this situation as evidenced by enhanced antibody response in animals treated with FCA alone or in combination with mebendazole as observed on day 56 of drug treatment. The increase in antibody titre is evidently due to FCA stimulating antibody production against antigens released from dead worms. The subsequent fall in titre as observed on day 91 might be due to lesser worm burden following death and elimination of parasites leading to lowered antigenic stimuli. Nevertheless, lowered immunostimulatory effect of FCA in due course cannot be ruled out.

The present observation thus indicates that suppressed immune status of filaria infected host can be reversed to a considerable extent by the administration of immunomodulators like FCA and this process itself can cause mortality of certain percentage of adult filariids. The increased macrofilaricidal activity of mebendazole in association with FCA thus appears to be immune mediated. Though 100 % adulticidal effect could not be achieved with combination therapy, a definite improvement in filaricidal action of mebendazole could be detected even by oral route.

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A DOMESTIC DUCK AS A NEW HOST FOR *BLASTOCYSTIS* SP.

Blastocystis hominis is a common inhabitant of the human intestine. However, *Blastocystis* sp. was also found in some animals: monkeys (McClure et al. 1980: Lab. Anim. Sci. 30: 890–899), pigs (Burden et al. 1978/1979: Vet. Microbiol. 3: 217–234, Pakandl 1991: Folia Parasitol. 38: 297–301), turkeys (Lee 1970: Trans. Br. Mycol. Soc. 54: 313–317), chickens and ostriches (Yamada et al. 1987: Parasitol. Res. 73: 527–531). It is questionable whether *Blastocystis* sp., in unrelated hosts, represents only one species.

We have found *Blastocystis* sp. in the caecum and cloaca of domestic duck (*Anas platyrhynchos f. domestica*) 60 days and 1 year after inoculation with the caecal content of mallards (*Anas platyrhynchos* L.). This experiment was carried out in order to propagate *Cochlosoma* sp. which occurs in the mallard. Using a method of cultivation in the biphasic egg-slant medium, supplemented by horse serum diluted in Locke's solution (Zierdt 1973: J. Protozool. 20: 114–121), we found *Blastocystis* in 3 out of 4 ducks. These cultures were processed for transmission electron microscopy using the previously described method (Zierdt, op. cit.).

The ultrastructure of these cells does not differ from the commonly known ultrastructure of *Blastocystis* (Tan and Zierdt 1973: Z. Parasitenkd. 42: 315–324, Dunn et al. 1989: Int. J. Parasitol. 19: 43–56). A large central body inside the cells was surrounded by a thin layer of cytoplasm, which is thicker only around the nuclei (Fig. 1a). The content of the central body was homogeneous, corresponding to the vacuolar form of the *Blastocystis*. The central body was usually elec-