Acanthocheilonema viteae in *Mastomys coucha*: Chemotherapeutic and Chemoprophylactic Role of Vitamin A in Experimental Filarial Infection

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The role of vitamin A was evaluated for its chemotherapeutic and chemoprophylactic action against *Acanthocheilonema viteae* infection in *Mastomys coucha.* Vitamin A was administered for 10 days, five days before infection and five days post infection. On day 0 experimental animals as well as controls were infected with L3, the infective stage. Establishment of the worms revealed significantly less percentage of worm recovery over untreated controls. Cell-mediated response was found to be the cause of this reduction in worm recovery, whereas humoral response was not significant as lgG, lgA and lgM titres were low.

Key words: Acanthocheilonema viteae, *Mastomys coucha*, Vitamin A

Introduction

Filariasis is a major parasitic disease of great public health importance affecting millions of people in tropical and subtropical countries (NICD, 1994). The disease is prevalent in developing countries where, along with parasitic infections, micronutrient deficiency is also a serious public health concern. Vitamin A deficiency is one of the major micronutrient deficiencies found in India (Chakravarty and Sinha, 2002). A direct correlation between protein-energy malnutrition, vitamin A deficiency and parasitic infections was found by Muniz-Junqueira and Queiroz (2002). Supplementation with vitamin A was found to potentiate host resistance to malaria, a well known parasitic disease. Apart from malaria, vitamin A supplements also had a significant effect on HIV-1 and diarrhea infections as well (Villamor *et al.*, 2002). Thus it was considered worthwhile to study the role of vitamin A in experimental filarial infection.

In the present study the chemoprophylactic and chemotherapeutic role of vitamin A has been investigated and after preliminary findings of a positive role of vitamin A against filariasis, the mechanism of action of the same was elucidated by studying the immunological role of vitamin A against the establishment of *Acanthocheilonema viteae* infection in *Mastomys coucha* (common name: golden rat).

Materials and Methods

Experimental model

The rodent filarial strain *Acanthocheilonema viteae* was transmitted to *Mastomys coucha* by injecting subcutaneously 50 L3 (infective larvae) obtained from the freshly dissected vector, *Ornithodorus moubata* (Bhatnagar *et al.*, 1995).

Experimental schedule

A batch of animals was divided into two groups. Group I received vitamin A (Roche Products Limited, Bombay) in two courses for five consecutive days once before infection and another after infection. A vitamin A tablet was dissolved in triple distilled water and was given in three doses, *i.e.*, 1000, 500 and 250 IU × 10. The first course was administered from day – 7 to – 2 before infection and the second from day + 7 to + 11 post infection. On day 0 both the groups were infected as above. At each dose level 8–12 animals were used in 3 replicates.

Recovery of adult worms: Animals were sacrificed under deep anesthesia on day 60 of infective larvae (L3) exposure and the worm burden was ascertained following the method of Bhatnagar *et al.* (1995).

Examination of uterine contents for parasites: Female parasites were teased on a glass slide and examined under a microscope to see any abnormality in developing stages and microfilariae.

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Statistical analysis

Data was analyzed by Student's T-test for determining *p*-values.

Immunological studies

A batch of male *Mastomys* was divided into two groups, one was kept as control and the other was experimental. Vitamin A treatment was given as in an earlier chemotherapeutic study. For enzyme-linked immunosorbent assay, blood was collected from the retro-orbital plexus of infected male *Mastomys* (control as well as experimental) on days 10, 30, 60, 90 and 120 post infection, kept at 37 °C for 2 h for serum to separate and centrifuged at 2000 rpm for 10 min. Clear serum was pipetted out and stored at −20 °C. On the 120th day animals were sacrificed and spleens were dissected out under sterilized conditions for the lymphocyte migration inhibition test.

Lymphocyte migration inhibition test

The assay was performed by the method of Singh *et al.* (1997). Briefly, spleens of two groups were excised and a splenocyte suspension was prepared after lysing red blood cells with 0.85% ammonium chloride (George and Vaughen, 1962; Bhatia *et al.*, 1981). Cell count was done using a haemocytomer and dilution was done so that each ml of cell suspension contained 5×10^6 cells. Cell suspension (5×10^6 cells) after three washings was drawn into capillaries (Laxbro, Bangalore, India). Incubation was done at 37 °C overnight. The area of cell migration was drawn on filter paper with the help of a camera lucida. The cell migration area was cut and weighed and compared with that of control animals.

Antigen preparation

Adult worms were recovered from the pleural cavities of heavily infected *Mastomys* and were washed with 0.85% saline dried between two layers of filter paper and homogenized for half an hour in ice. The homogenate was sonicated at 10 KCS for 15 min. To the antigen, merthiolate was mixed in a ratio of 1:10,000. Protein content was estimated according to Lowry *et al.* (1951).

The enzyme-linked immunosorbant assay was done according to Voller *et al.* (1979).

Results

Worm recovery

The establishment of the rate of adult *Acanthocheilonema viteae* was lower in the experimental group in comparison to that of control groups. There was 68.2, 51.5 and 47.5% reduction in the worm recovery over controls at doses of 1000, 500 and 250 IU × 10 (Table I). Female worms showed a dose-dependent response, their percentage reduction over controls was 65.4, 47.6 and 39.9%, while in males there was no dose-dependent response at lower doses.

Uterine contents

All surviving females contained normal and active developing stages in their uteri.

Immunological response

There was a variation in the lgG, lgM and lgA titres amongst controls and experimental animals (Figs. 1, 2 and 3). Values of the lgG titre (Fig. 1) were higher in case of untreated controls over the experimental animals but they followed the same

Table I. Adult worm recovery from animals following treatment with vitamin A.

Experimental Group	Dose (IU × 10)	Number of worms recovered (% reduction over control)			Decrease in worm recovery
Treated		Female	Male	Total	
	1000	3.75 ± 1.8 (65.4)	3.25 ± 1.2 (71.3)	7.0 ± 1.6 (68.2)	3.14**
	500	5.67 ± 1.2 (47.6)	5.0 ± 0.9 (55.9)	10.67 ± 1.8 (51.5)	2.06*
	250	6.5 ± 2.1 (39.9)	5.0 ± 1.4 (55.9)	11.5 ± 0.7 (47.5)	1.9*
Control	Vehicle	10.83 ± 2.1	11.3 ± 3.8	22.0 ± 3.7	1.0

* $p < 0.01$, significant; ** $p < 0.001$, highly significant.

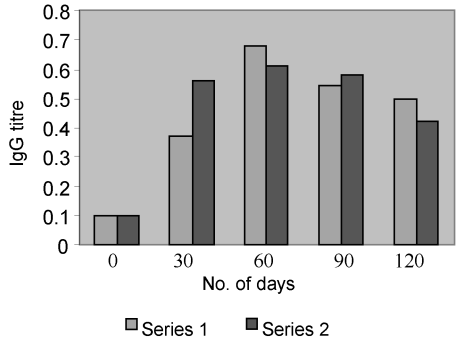

Fig. 1. Bar diagram of lgG titre versus no. of days. Series 1, untreated infected controls; series 2, vitamin A treated infected animals. Range of standard deviation was 0.008 to 0.012

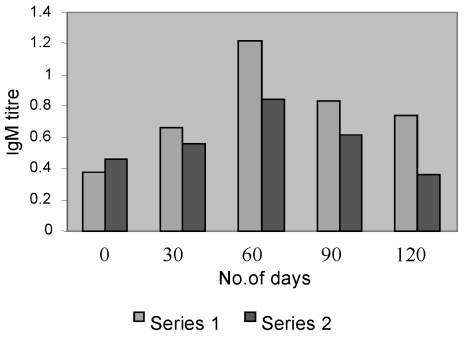

Fig. 2. Bar diagram of lgM titre versus no. of days. Series 1, untreated infected controls; series 2, vitamin A treated infected animals. Range of standard deviation was 0.01 to 0.03.

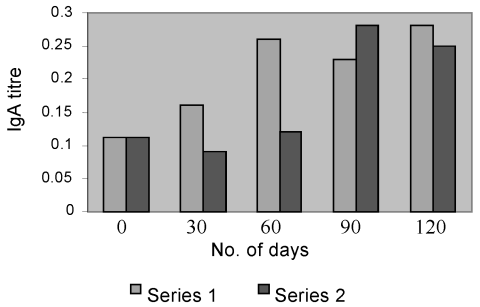

Fig. 3. Bar diagram of lgA titre versus no. of days. Series 1, untreated infected controls; series 2, vitamin A treated infected animals. Range of standard deviation was 0.008 to 0.031

trend, *i.e.*, lower at 30th day highest at day 90 and later a gradual decline. The trend in the lgM titre (Fig. 2) was similar, only on day 30 the titre of lgM in treated animals was higher as compared to controls, but there was a remarkable difference in control and treated on day 90. Regarding the lgA titre (Fig. 3) no clear trend was followed. Lymphocyte migration in all the sets was significantly enhanced in vitamin A supplemented animals (Table II).

Table II. Lymphocyte migration test in vitamin A treated animals and control animals.

Set no.	Treatment	Lymphocyte migration (weight of filter paper in g)	Increase in migration over controlled animals (%)
Set I	Control	0.43 ± 0.1	
	Treated	1.27 ± 0.23	66.1
Set II	Control	0.44 ± 0.15	
	Treated	1.20 ± 0.11	63.4
Set III	Control	0.41 ± 0.08	
	Treated	1.42 ± 0.14	71.2
Set IV	Control	0.56 ± 0.13	
	Treated	1.55 ± 0.24	63.9

Discussion

A number of studies has been conducted and support the view that the status of vitamin A has a significant role in resisting the infection and diseases (Cohen and Elin, 1974; Hof and Emmerling, 1979). The same was found in the case of experimental filarial infection. Supplementation of vitamin A before and after the infection resulted in the decrease of worm recovery in a dose-dependent manner. lgG response in experimental vitamin A treated animals was higher on day 30 as compared to the infected animals as controls. However on day 60 infected controls had a higher lgG titre which reduced gradually. This could be due to the enhancing of lgG response in the initial phase in treated animals for the control of filarial infection and later there was a gradual decline. Earlier response of lgG in vitamin A treated animals is in accordance with the studies of Smith and Hayes (1987), while in normal infected controls the lgG titre was at its peak on day 90 and followed by decline.

According to some of the studies (Bloomhoff *et al.*, 1992; Ross, 1992) vitamin A and its derivatives have been reported to enhance cell-mediated response as well as humoral responsiveness, same was observed in case of cell-mediated response in the form of increased lymphocyte migration but

the overall picture in case of humoral response of lgG, lgA and lgM titre was not conclusive.

The present study has in fact confirmed the role of vitamin A as a chemoprophylactic agent, but even at its highest dose it could not eradicate the parasitic worm burden but was successful in reducing the establishment of the parasitic infection to 60%. This study has indeed provided a lead in the form of vitamin A's role in filariasis, and in combination with other standard drugs like diethyl carbamazine and ivermectin, it might be able to combat the filarial menace, but requires further studies in the same direction.

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