Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 35 (1978)
Heft: 1

Artikel: Alteration of amicrofilaremia in "Dipetalonema viteae" infected hamsters with immunosuppressive drugs
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DOI: https://doi.org/10.5169/seals-312370

Acta Tropica 35, 57–61 (1978)

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Alteration of amicrofilaremia in *Dipetalonema viteae* infected hamsters with immunosuppressive drugs

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Summary

Dipetalonema viteae-infected hamsters with amicrofilaremic infections were subjected to immunosuppressive therapy. Methyl prednisolone acetate caused the most severe recrudescence of microfilariae while cyclophosphamide caused a low level, transient microfilaremia. Saline injected control hamsters remained amicrofilaremic. Neither drug influenced the number of adult worms recovered at necropsy in the treated hamsters compared with control hamsters.

Key words: D. viteae; microfilaremia; immunosuppression.

Introduction

Several experimental host-filarial worm systems exhibit remission of microfilaremias (amicrofilaremic, cryptic, occult or latent infections). The absence of peripheral microfilaremias with concomitant adult worm infections has been noted in infections with *Dirofilaria immitis* in the dog (Wong et al., 1973), *Litomosoides carinii* in the albino rat (Ramarkrishnan et al., 1962) and *D. viteae* in the golden hamster (*Mesocricetus auratus*) (Weiss, 1970; Neilson and Forrester, 1975). Bagai and Subrahmanyam (1970) administered cortisone to *L. carinii* infected albino rats which were amicrofilaremic and detected circulating microfilariae in these animals within a week of the initiation of cortisone therapy.

The present experiments were designed to investigate the effect of immunosuppressive agents, a corticosteroid (methyl prednisolone acetate) and an alkylating agent (cyclophosphamide) upon amicrofilaremic infections of *D. viteae* in hamsters.

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Material and methods

Parasite and infection

Random bred male hamsters (Lak/LVG, Lakeview Hamster Colony, NJ), eight weeks of age at the start of the experiments, were each given a single infection of 100 *D. viteae* larvae subcutaneously. The animals were treated with immunosuppressive agents or saline beginning on day 150 postinfection.

Hamsters of similar age, sex and strain were given ten infections each of 50 larvae at two week intervals. Treatment of these multiply infected hamsters with immunosuppressive agents or saline was begun 87 days after the final infection. This was day 150 after the first infection.

Details of the techniques for isolation of larvae and infection of hamsters have been described (Neilson and Forrester, 1975).

Treatments

The singly and multiply infected hamsters were each divided into three groups making a total of six groups with six animals in each group. One group of both singly and multiply infected hamsters received methylprednisolone acetate (MP) (Depo-Medrol, Upjohn) at a dose rate of 3 mg/kg body weight injected intramuscularly twice weekly for 3 weeks. Likewise a group of both singly and multiply infected hamsters received cyclophosphamide (CY) (Cytoxan, Mead Johnson) in saline twice weekly for 3 weeks at a dose rate of 150 mg/kg body weight intraperitoneally. The remaining two groups served as controls and received injections of saline.

Microfilariae and adult worm counts

Fifty mm^3 blood samples were taken from the retro-orbital sinus of each animal at weekly intervals before treatment and twice weekly after treatment and examined for microfilariae. The techniques used for staining and counting microfilariae and collecting and counting adult worms at necropsy on day 205 from the start of the experiments, have been described (Neilson and Forrester, 1975).

Results

Both the singly and multiply infected hamsters developed microfilaremias following infection with larvae. The average peak microfilaremias observed between day 70 and 80 postinfection were 1900 and 2500 mf/ml blood for the singly and multiply infected groups respectively. These levels were followed by a steady decline in microfilaremia, the singly and multiply infected groups reaching zero at 110 days and 120 days postinfection respectively.

As summarized in Table 1 it is evident that both drug regimens allowed the establishment of peripheral microfilaremias in both infection groups. Microfilariae were detected four to seven days after the initiation of treatment. The onset and degree of microfilaremia was similar in both the singly and multiply infection groups given CY. MP induced a low but more prolonged microfilaremia in the singly infected animals. MP treated multiply infected hamsters displayed the most persistent and severe microfilaremias. No microfilariae were detected in the blood of the saline injected control hamsters during this period.

At necropsy on day 205 from the start of the experiment, the mean ± standard deviation adult worm numbers of the singly and multiply infected

Table 1. Number of *Dipetalonema viteae* microfilariae per ml blood in hamsters given single or multiple infections and treated with methyl prednisolone acetate (MP), cyclophosphamide (CY) or saline. Mean number of *D. viteae* microfilariae per ml blood*

Day from start of experiments	MP		CY	
	single infection	multiple infection	single infection	multiple infection
150△	0□	0	0	0
152△	0	0	0	0
154△	0	50	0	0
157△	20	450	20	60
159△	60	1050	40	40
161△	20	800	20	20
164	110	1200	60	80
166	120	900	40	20
168	40	800	0	0
171	20	1100	0	0
173	0	800	0	0
175	0	400	0	0
178	0	80	0	0
180	0	200	0	0
182○	0	0	0	0

* Both the singly and multiply infected hamsters injected with saline had negative microfilariae counts between day 150 and 185.
△ Denotes days on which the appropriate drug treatment was given.
□ Each value represents the mean of 6 animals.
○ All groups had negative blood microfilariae counts from day 182 until necropsy on day 205.

hamsters were 25 ± 9 (14 males + 11 females) and 74 ± 26 (42 males + 32 females) per hamster, respectively. The adult worm burdens within each infection group were similar regardless of drug or saline treatment. No subcutaneous nodules were seen in the singly infected group, however, the multiply infected hamsters had an average of 23 nodules per hamster. Previous work (Neilson, 1976) has shown that each subcutaneous nodule results from the entrapment of a single worm. When the adult worm burdens plus nodules were expressed as percentages of the total number of infective larvae given, values of 25% and 19% were obtained for the singly and multiply infected hamsters respectively.

Discussion

The present work demonstrates that the amicrofilaremic state which develops in *D. viteae* infected hamsters can be altered by the administration of immunosuppressive agents. The steroid, MP, induced the most extensive reappearance of microfilariae, however, this level was considerably lower than those which developed soon after patency in both the singly and multiply infected

hamsters. There was no correlation between the number of female worms and the microfilaremia induced. For instance, the MP treated multiply infected hamsters had about 10 times as many microfilariae in their blood compared to similarly treated single infection hamsters. At necropsy the former group of hamsters had only three times as many adult female worms compared to the latter.

CY treatment induced similar transient low level microfilaremias in both singly and multiply infected hamsters. At the levels used in the present experiment, CY was a less efficient immunosuppressive agent than MP insofar as resistance to *D. viteae* in hamsters is concerned.

The levels of the two drugs used were chosen for the following reasons. Golden hamsters tolerate well the daily administration of corticosteroids at a dose rate of 3 mg/kg body weight (Frenkel and Havenhill, 1963) and such treatment increased the susceptibility of this species to infection with *Necator americanus* (Sen and Deb, 1973).

CY at a dose rate of 10 mg/kg body weight given twice weekly suppressed the development of immunity in hamsters to *Toxoplasmosis* (virulent RH strain), however, this drug regimen suppressed an established immunity to this organism in only one of six hamsters (Frenkel et al., 1975). Hamsters became highly susceptible to *Besnoitia* infections when treated with CY at either 10 mg or 15 mg CY/kg body weight thrice or twice weekly respectively (Wilson and Frenkel, 1971). This report states that CY at 15 mg/kg body weight caused toxicity in uninfected hamsters. Moolten et al. (1975) found no drug related toxicity in hamsters given up to four doses of 180 mg CY/kg body weight at weekly intervals. This dose rate depressed the neutralizing antibody response of hamsters to parenterally administered y-globulin-diphtheria toxin conjugate. CY given during the course of hamster immunization ablated the primary response to this antigen and completely suppressed the secondary response. Guinea pigs given 30 daily doses of 15 mg CY/kg body weight failed to expel adult *Trichinella spiralis* from the intestine (Martynowicz, 1975). These studies indicated that this level of treatment, a total of 450 mg CY/kg, effectively inhibited development of immediate and delayed-type hypersensitivities.

Hamsters tolerated well a total of 720 mg CY/kg body weight given over a four week period (Moolten et al., 1975). Hamsters in the present experiment received a total of 900 mg CY/kg body weight over a 3 week period. It was anticipated that this dose rate would severely diminish both cellular and humoral immune responses. There is evidence to suggest that the amicrofilaremic state in *D. viteae* infected hamsters may be due to an antibody mediated response (Weiss, 1970). Clearly, in the present work, CY had only a slight suppressive effect upon this response when compared to the effect thereon of MP.

When hamsters are given multiple (trickle) infections similar to those in the multiple infection group of the present experiment, approximately 20% of each dose of infective larvae reach the adult stage (Neilson, 1976). The final adult

worm burden, therefore, arises from the cumulative sum of 20% of each dose of larvae reaching maturity. Some unknown proportion of the 80% of each larval dose unaccounted for, might be present in the host as inhibited immature forms. Attempts made so far to detect immature larval forms have been negative (Neilson and Forrester, 1975), however as such stages are very difficult to find, their existence remains a possibility. If such inhibited forms exist, immunosuppression of the host might have allowed there normal development. This would have been reflected in higher adult worm burdens especially in the MP treated multiply infected hamsters. No such increase was found and while this is not absolute proof of the absence of inhibited forms, it lends credence to the supposition that such forms do not exist.

Acknowledgments. I thank M. L. Boyette and J. M. Dale for their valuable technical assistance. The strain of *D. viteae* used was obtained from the Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, Georgia under the auspices of the US-Japan Cooperative Medical Science Program, NIAID. This study was supported by the US-Japan Cooperative Medical Science Program Administered by NIH, Grant AI 10803. Florida Agriculture Experiment Stations Journal Series No. 402.

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