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Experimental infection of *Meriones unguiculatus* with subperiodic *Brugia malayi*: some parasitological observations

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ABSTRACT The *Meriones unguiculatus-Brugia* spp. model is used extensively for experimental studies and for the primary screening of potential filaricides. The parasitological aspects of this model were studied to determine the optimal conditions for maintaining parasites, screening of drugs, and for studies on host-parasite relationships.

At autopsy 200 days post-infection, the mean±SD worms recovered from female and male jirds infected with 50 L3 were not statistically significantly different, these being 5.1±6.9 and 8.1±9.2, representing 10.2% and 16.2% of the infective dose, respectively. In those given 100 L3, worm recovery from females and males was also not significantly different, these being 20.8±33.5 and 8.5±5.7 respectively. Recovery of worms was much higher when animals were infected by the intraperitoneal (IP) compared to the subcutaneous (SC) route. In those infected with 50 L3, the worm recovery was 15% and 8% by the IP and SC routes respectively. The corresponding figures for animals given 100 L3 were 21% and 3% respectively. Of the 286 worms recovered, 46% and 54% were females and males, respectively. In IP infected animals, 80% of the worms were recovered from the peritoneal cavity. In those infected SC, 30% and 33% were recovered from the heart and lungs respectively.

The prepatent periods in those given 50 L3 IP were 172.9±42.3 and 126.3±32.1 days in female and male jirds, respectively, and in those given 50 L3 SC were 152.6±44.4 and 150.8±45.3 days, respectively. Microfilarial density increased rapidly to a peak at 8-12 weeks from patency and then leveled off at about 40-50 mf/20 µL in those infected SC, whereas counts were very low in those infected IP. The periodicity indices and peak hours in IP and SC infected animals were 33.4 and 10.04 hrs, and 67.6 and 7.88 hrs, respectively. The appropriate infective dose for infection of jirds for use in the screening of filaricides is 50 L3 either IP or SC.

ABSTRAK Aspek-aspek parasit model *Meriones unguiculatus-Brugia* spp. telah dikaji untuk menentukan keadaan-keadaan optimum untuk mengkultur parasit, mengenalpasti drug-drug dan mengkaji perhubungan hos-parasit.

Semasa autopsi pada 200 hari post-jangkitan, bilangan min±SD cacing dewasa yang didapati dari jird-jird betina dan jantan yang dijangkiti dengan 50 L3 tidak berbeza dengan signifikan secara statistik, iaitu 5.1±6.9 dan 8.1±9.2, yang mewakili 10.2% dan 16.2% dos jangkitan masing-masing. Bilangan cacing dewasa yang didapati dari jird-jird betina dan jantan yang dijangkiti dengan 100 L3 juga tidak berbeza dengan signifikan, iaitu 20.8±33.5 dan 8.5±5.7 masing-masing. Jumlah cacing yang didapati dari binatang yang dijangkiti secara intraperitoneal (IP) adalah lebih banyak daripada yang dijangkiti secara subkutanus (SC). Bagi kajian yang menggunakan 50 L3, 15% dan 8% dos jangkitan didapati sebagai cacing dewasa masing-masing. Untuk kajian yang menggunakan 100 L3, nilai ini adalah 21% dan 3% didapati masing-masing. Daripada 286 cacing yang dijumpai, 46% dan 45% adalah betina dan jantan masing-masing. Bagi binatang yang dijangkiti secara IP, 80% cacing dijumpai di rongga peritoneal sementara jangkitan yang dibuat secara SC, 30% dijumpai di jantung dan 33% di paru-paru.

Tempoh pripaten bagi binatang betina dan jantan yang dijangkiti dengan 50 L3 IP ialah 172.9 ±42.3 hari dan 126±32.1 hari, masing-masing, manakala secara SC nialainya adalah 152.6±44.4 dan 150.8±45.3 hari, masing-masing. Kepadatan mikrofilaria (mf) naik dengan cepatnya hingga ke paras tertinggi pada 8-12 minggu dari patensi dan kemudian menurun ke paras 40-50 mf/20 µL bagi jangkitan secara SC. Bagi kajian menggunakan kaedah IP nilainya amat rendah. Indeks periodisiti dan masa kemuncak di binatang yang dijangkiti IP adalah 33.4 dan jam 10.04 pagi, dan secara SC nilainya adalah 67.6 dan jam 7.88 pagi. Dos infeksi yang sesuai bagi jangkitan jird yang diguna untuk ujian ubat filarisid ialah 50 L3 secara IP atau SC.

(filariasis, *Brugia malayi*, parasitology, gerbil)

INTRODUCTION

The subperiodic *Brugia* spp.-*Meriones unguiculatus* model [1, 2] has certain advantages over other experimental models of lymphatic filariasis for the study of host-parasite relationships, and production of various parasite stages as antigen source for immunodiagnosis and immunological studies. Adult worms and microfilariae are easily harvested from the peritoneal cavity if the animal is intraperitoneally inoculated with infective larvae (L3). It has also certain advantages when used for the primary screening of potential filaricides; the jird being a small animal, is easy to maintain and is highly susceptible to the parasite. It is also easy to monitor the effect of drug treatment on the various stages of the parasite. In addition, naive jirds implanted intraperitoneally with a defined number of adult worms, have been used for primary screening of filaricidal compounds [3, 4]. However, to optimise this model for the screening of drugs or for other experimental studies, it is necessary to determine basic features of the host-parasite relationship like the pattern of microfilaremia over time, the microfilarial periodicity, and the worm load after diffe-

rent routes of infection. In the present study, these parasitological parameters in jirds experimentally infected with 50 or 100 L3 either SC or IP were investigated.

MATERIALS AND METHODS

Subperiodic *B. malayi* L3 were harvested through mass dissection in RPMI-1640 medium of *Aedes togoi* fed two weeks previously on microfilaremic leaf-monkeys (*Presbytis cristata*) maintained at the Institute for Medical Research.

Jirds (*Meriones unguiculatus*) were inoculated with 50 or 100 L3 of subperiodic *B. malayi* either SC or IP. In the first study 19 and 5 jirds were infected IP with 50 and 100 L3, respectively. Another 6 and 3 jirds were infected with 50 and 100 L3 SC. Infected jirds were examined for microfilaremia using 20 µL blood obtained from the tail vein, starting from 8 weeks postinfection. Autopsy of the animals was carried out at about 200 days postinfection to determine the adult worm load. At autopsy the peritoneal and thoracic cavities and all organs were examined for adult worms using the method of Buckley and Edeson [5]. The carcass was also soaked in normal saline for an hour to recover any other worms left behind.

In another study, 37 and 31 jirds were infected IP and SC, respectively, and the pattern of microfilaremia observed over time. Of these 68 jirds, 51 (30 ♀, 21 ♂) and 17 (8 ♀, 9 ♂) were infected with 50 and 100 L3, respectively. The periodicity of the microfilaremia was also determined in 10 microfilaremic jirds infected SC (5) and IP (5) from microfilarial counts of blood collected at 4 hourly intervals, according to the method of Aikat and Das [6] as modified by Tanaka [7].

RESULTS

Thirty three (15 ♀, 18 ♂) jirds were injected intraperitoneally (IP) or subcutaneouly (SC) with subperiodic *B. malayi* infective larvae (L3). Nineteen and 5 were IP infected with 50 and 100 L3, respectively, while 6 and 3 were SC infected with 50 and 100 L3, respectively. Autopsy of IP and SC infected animals were carried out at 201.9±78.7 and 199.3±55.7 days post-infection, respectively. The mean±SD worms recovered from female and male jirds given an infective dose of 50 L3 were not statistically significantly different, these being 5.1±6.9 and 8.1±9.2, representing 10.2% and 16.2% of the infective dose, respectively. In those given 100 L3, worm recovery from females and males was also not significantly different, these being 20.8±33.5 and 8.5±5.7, respectively. Recovery of worms was much higher when animals were infected by the IP route. In those infected with 50 L3, the worm recovery was 15.4% and 7.7% by the IP and SC routes, respectively. The corresponding figures for animals given 100 L3 were 21.4% and 3.3% respectively. There was no significant difference between the number of male and female worms recovered, 132 (46.2%) and 154 (53.8%) out of 286 worms being females and males, respectively. In IP infected animals, 203 out of the 253 (80.2%) worms recovered were from the peritoneal cavity. In those infected SC, 10 (30.3%) and 11 (33.3%) of the 33 worms recovered were from the heart and lungs, respectively. Another 68 (38 ♀, 30 ♂) jirds were infected IP (37) and SC (31) and the microfilaremia followed up. Of these, 51 (30 ♀, 21 ♂) and 17 (8 ♀, 9 ♂) jirds were infected with 50 and 100 L3, respectively. The prepatent periods in those given 50 L3 IP and SC were 172.9±42.3 and 126.3±32.1 days, respectively; in females and males these were 152.6±44.4 and 150.8±45.3 days, respectively. Microfilarial density increased rapidly to a peak at 8-12 weeks from patency and then leveled off at about 40-50 mf/20 µl in those infected SC, whereas counts were very low in those infected IP (Fig. 1). The periodicity indices and peak hours in IP and SC infected animals were 33.4 and 10.04 hrs, and 67.6 and 7.88 hrs respectively (Fig. 2).

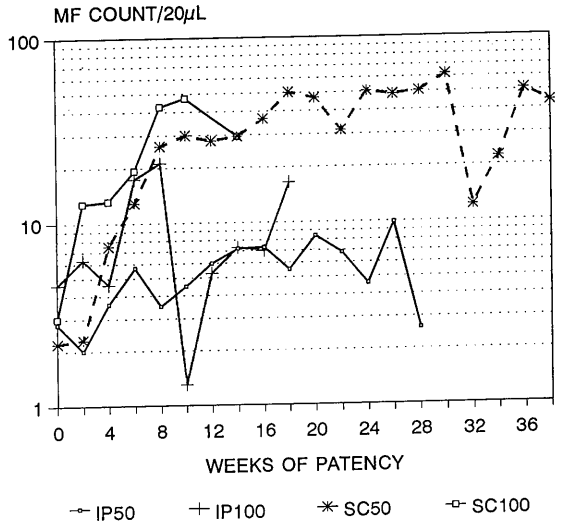

Figure 1. Microfilaremia in *Meriones unguiculatus* infected with 50 or 100 infective larvae of subperiodic *Brugia malayi* given intraperitoneally (IP) or subcutaneously (SC).

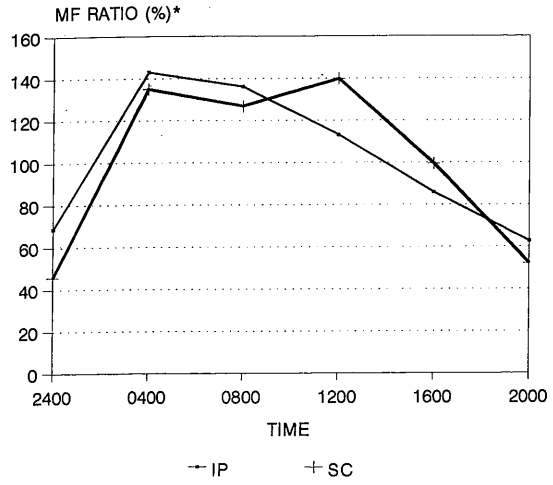

Figure 2. Microfilaremia over 24 hours in *Meriones unguiculatus* infected intraperitoneally (IP) or subcutaneously (SC) with subperiodic *Brugia malayi* infective larvae.
*MF ratio (%) = microfilarial count/mean microfilarial count (%); average ratio of 5 animals at each time point.

DISCUSSION

The jird has been shown to be a good host for *B. malayi* or *B. pahangi* [1, 2] and IP or SC infected animals have been used for the screening of potential filaricides [8, 9]. Naive jirds intraperitoneally implanted with adult *Brugia* parasites have also been used to screen for adulticidal activity of compounds [3]. This latter model is perhaps not as useful as L3 infected jirds for determining the filaricidal activity of compounds as there is no contributory effect of host immunological responses on treatment, as would be expected in naturally acquired infections.

The present findings show that the mean prepatent period is about 150 days and is earlier in SC compared to IP infected jirds. This is probably due to the low microfilarial density in the blood of IP infected animals as most microfilariae accumulate in the peritoneal cavity. As more than 80% of the worms are also localized in the peritoneal cavity of IP infected jirds, this route of infection would allow easy monitoring of drug effect on the adult parasites. However, it is uncertain whether compounds for evaluation should be injected directly into the peritoneal cavity or subcutaneously. If the former route is used, an abnormally high concentration of the unmodified compound in the peritoneal cavity may provide effects different from that by the subcutaneous or oral routes. Although 63% of the adult worms in SC infected jirds were localized in the heart and lungs, the IP infected jird would still be a more appropriate model for testing the adulticidal activity of oral or subcutaneously injected formulations, as it is easier to recover worms from the peritoneal cavity. The SC infected jird would be a better model for the screening of microfilaricidal activity of compounds as microfilaremia is very much higher when compared to that in IP infected animals. In this respect, it is important to note that peak microfilaremia is attained at 8-12 weeks from patency and that the periodicity is subperiodic in pattern with the peak count at about 08.00 hrs. An infective dose of 50 L3 given either IP or SC, appears to be appropriate for infection of jirds used in screening of filaricides.

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