FULL PAPER *Parasitology*

Migration of *Strongyloides venezuelensis* in Rats after Oral Inoculation of Free-Living Infective Larvae

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(Received 15 August 2002/Accepted 8 May 2003)

ABSTRACT. *Strongyloides venezuelensis* (SVZ) infection was chronologically monitored in 85 Sprague-Dawley rats (SDR), which were orally inoculated with approximately 1,000 infective larvae. In order to describe the characteristics of migrating larvae (MLS) in various visceral organs (the liver, lung, cardiac blood, and small intestine), 5 SDR were sacrified at 20 min, 45 min, 1 hr, 2 hr, 3 hr, 4 hr, 8 hr, 12 hr, 16 hr, 48 hr, 72 hr, 96 hr, 120 hr, 144 hr, 168 hr and 192 hr post inoculation (PI). MLS were recovered from the liver a nd blood 20 and 45 min PI and measured 788 ± 26 μm and 846 ± 40 μm in length, respectively. MLS were first observed in the lung tissue 45 min PI and measured 925 ± 38 μm on the average. In the trachea, MLS measuring 849 ± 75 μm appeared 3 to 96 hrs PI. Adult worms (AWS) measuring 1,926 ± 521 μm to 2,956 ± 159 μm in length were observed in the small intestine from 120 hr PI. The worms appeared to mature more than 168 hr PI and attained the average maximum length of 2,420 ± 532 μm. At 3 hr PI focal hyperemic and necrotic lesions were evidently observed in the liver and lung, together with eosinophilic infiltration in the stomach, liver, and lung. The parasites were histologically detectable in the lung tissues but were very difficult to find in the liver and the epithelial layer of small intestine. These data demonstrate that SVZ parasites take 20 min to reach the liver via the stomach and only three hours to reach the trachea through the same route. The development from eggs to adults takes 168 hr in the SDR model.

KEY WORDS: free living parasite, life cycle, migration route, parasite, *Strongyloides venezuelensis*.

J. Vet. Med. Sci. 65(9): 971–975, 2003

Strongyloides venezuelensis (Brumpt, 1934) (SVZ), a parasitic nematode of rodents, has been used recently as a suitable parasite model for the studies of biology [21, 27, 30, 32], immunology [8, 11, 16], morphology [9, 12, 32], biochemical characteristic [31, 33], worm expulsion mechanism [2, 13], and nematocidal activity [7, 20, 22, 28]. Our previous studies on SVZ have been made to establish the foundation of an *in vitro* cultivation technique [1, 3] and described that Mongolian gerbil was a suitable carrier animal model for persistent infection [4].

SVZ has been reported from different parts of the world [10, 34]. The infection of rodents with SVZ has been carried out in various species such as rats [34], mice [15, 21, 27], Mongolian gerbils [30], Syrian golden hamster [24], and Wistar Fischer rat [2, 27] for various purposes and protocols of research.

This strongylid invades the host by skin penetration, migrates to the lungs where it molts to the 4th stage larvae, and then travels to the small intestine where it finally molts to mature adults [5, 29, 35]. The morphologic and kinetic time-lines, however, have not been fully elucidated. Takamure [27] observed intracorporal distribution of migrating filarial parasites (MFP) in Wistar rats and ddy mice from 24 to 96 hr after subcutaneous inoculation, and reported that the MFP were found in the subcutaneous tissue and muscles of rats for 42 hr after exposure. He also described the phenomenon of "concentration" of MFP in the lungs from 45 hr PI adult worms (AWS) appeared in the small intestine just 60 hr PI. On the contrary, the intracorporal migrating course of MLS has not been fully described in orally inoculated animals except the observation that the recovery rate of AWS was lower in the case of oral than in subcutaneous or percutaneous inoculation [26].

Strongyloidiasis is a scarcely studied parasite that infects no fewer than 100 million people worldwide, generally in the regions between latitudes 35°N and 30°N. The disease is predominantly distributed in warm moist areas because such climates are suitable for the survival of the larval stage [14, 18]. The soil-transmitted nematode, SVZ, occurs in two developmental stages: the free living and parasitic stages. When SVZ infects various species of animals (*Mesocricetus auratus*, *Phodopus campbelli*, *Cricetulus griseus*, *Tscherskia triton*), the quantity and peak of egg production differs from host to host [23].

The present study was conducted to determine the duration taken by SVZ to reach various internal organs, specially the heart, liver, lung, trachea and small intestine and thereafter, the development of larva in these organs after oral inoculation of free living filarial larval forms (L_3) in the Sprague-Dawley rats (SDR) model over a period of 192 hr PI. Histopathological observation was also used to confirm the route and distribution of migrating larvae (MLS) in each internal organ.

MATERIALS AND METHODS

Animals: All the experiments were carried out with male inbred SDR, purchased from the Korean Chemical Research Institute, bred by sib-mating and raised under conventional

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laboratory conditions. Eighty five male animals were 8 weeks old and weighed 150–180 g. Autoinfection was minimized by maintaining a clean environment, and housing the animals in stainless steel raised cages with 5 animals each. The cages were power-washed twice a day. Animals were allowed *ad libitum* to feed and drink water throughout the period of the experiment.

Parasites: *S. venezuelensis* (Kogoshima isolate) was a generous gift from Dr. H. Saeki, Nippon Veterinary and Animal Science University, Tokyo, Japan. The worms were maintained by serial passage through Mongolian gerbils over a period of 5 years according to the previously described protocols [25]. Infective larvae of SVZ were prepared and standardized as previously described [1]. Briefly, L_3 were prepared from fecal cultures in polyvinyl bags (4 × 10 cm) each containing a cotton plug (500 mg) soaked in suspension of feces (1.0 g) in distilled water (20 m*l*). For inoculation, L_3 were washed three times in sterile saline (SS) containing 200 units/m*l* penicillin (Sigma, St. Louis, U.S.A.) and 200 units/m*l* of streptomycin (Sigma, St. Louis, U.S.A.). The L_3 suspension was prepared in SS (1,000 larvae/m*l*) immediately after their viability was ascertained.

Inoculation of L_3 into the stomach: After over-night fasting, the animals were orally inoculated with 1,000 L_3 in 1 m*l* SS by intragastric intubation. Five animals were sham-inoculated with saline serving as normal controls.

Recovery of MLS and AWS in the internal organs: The course of migration of parasite was monitored through the appearance of larval stages in each target site. Total fecal egg counts and sequential measurements of parasite length were recorded. At the designated time interval of examination, five animals per group were sacrificed using an anesthetic overdose of ether. The criterion was to pin-point the first appearance and stay of the parasite in the blood, liver, lung or small intestine. Serial histological samples were also collected and preserved until examined. The MLS and/or AWS were monitored in the blood, liver, trachea, lung, and small intestine of each animal 20 min, 45 min, and 1 hr, 2 hr, 3 hr, 4 hr, 8 hr, 12 hr, 16 hr, 48 hr, 72 hr, 96 hr, 120 hr, 144 hr, 168 hr, and 192 hr PI, as previously described [34]. In order to recover MLS from blood, blood samples were collected through cardiac puncture under ether-anesthesia, hemolyzed with sterile tap water, and examined under a light microscope for the presence or absence of MLS. The pharynx and trachea were removed immediately after sacrified and ligated with a fine forceps. The trachea was filled with 1 m*l* of warm SS and incubated at room temperature for 30 min prior to examination of larval motility. MLS were then recovered by a low speed centrifugation. The liver and lung samples were then removed, minced to small pieces in warm SS, and transferred to the Baermann system filled with 50 m*l* of SS. After 30 min incubation at room temperature, the vigorous motility of MLS was recorded in minced liver and lung samples. Definitive counting was not attempted since most samples contained so many MLS to count. AWS were recovered from the small intestine by longitudinal opening and careful scraping of mucosal surface. The scrapied mucous material was placed in a petri-dish containing SS (37°C), incubated for 10 min by gentle shaking, and then larvae were collected with the Baermann apparatus. The MLS were allowed to settle down on the bottom of petri-dish, counted by light microscopy and recorded separately as immature and mature.

Morphological observation of MLS and AWS: The body length of MLS and AWS recovered from each organ was measured under a light microscope. Egg formation in the ovary was also determined among AWS as an index of fecundity.

Histopathological observation: Two of the 5 sacrificed animals at each time interval were subjected to pathologic examination. The tissues (approximately 1.0 cm in length) from the stomach, small intestine, liver, and lung were fixed in 10% buffered formalin, dehydrated, cleared in absolute alcohol/xylene and embedded in paraffin according to a standard protocol. Histopathological sections were cut in 4 μm thickness, and stained with hematoxylin-eosin prior to examination.

RESULTS

Appearance and disappearance of parasites in the internal organs: The vigorously moving MLS were observed in the minced liver samples 20 min PI and disappeared by 3 hr PI (Table 1). The MLS in the cardiac blood and lung were first observed 45 min and 3 hr PI, respectively, but no larvae was observed in the blood and lung tissue after 3 hs and 72 hr PI, respectively. A typical 4th larva (L_4) (Fig. 1) was recovered from the lung. This larva showed very vigorous movement, exemplifying the classical "spiral movement". The MLS were recovered in the trachea 3 to 96 hr PI, when they showed strikingly vigorous movement. An immature adult worm which were obtained from the small intestine from 120 hr PI, kept indistinct eggs in uterus (Fig. 2). Well developed eggs were observed in the uterus in mature adults from 144 hr PI but no rhabditiform larvae were detected from both the intestinal mucous and feces.

Body length of MLS and AWS collected: The average of body lengths (Mean ± SD) of MLS and AWS from each organ throughout the observation period are summarized in Table 1. The MLS from the blood and lung measured 846 ± 40 μm and 849 ± 75 μm, respectively. The MLS from the trachea were 925 ± 38 μm in body length, and was significantly longer than those recovered from the liver and lung. The immature females in the small intestine averaged 2,278 ± 679 μm in length 144 hr PI (Table 2), and well developed eggs were evidently observed 168 hr PI. The length and width of adult parasites, recovered 192 hr PI, was 2,956 ± 159 μm and 31.5 ± 28.0 μm, respectively.

Histopathologic observation: Grossly, no significant lesions were observed on the wall of the stomach and small intestine. Microscopically, lesions caused by MLS could not be demonstrated except eosinophilic infiltration in the lamina propria, especially near lamina subglandularis of the stomach and small intestine 1 hr PI. The livers had a mot-

Table 1. Time of appearance and disappearance of migrating larvae (MLS) and adult worms from each organ of Sprague-Dawley rats after oral inoculation with 1,000 free-living larvae of *Strongyloides venezuelensis*

Organs	Time of		Length (μm) of parasite (Mean ± SD)	Number of parasite
	First-A PI[c]	Dis -A PI[d]		
Liver[a]	20 min	3 hr	788 ± 26	50 ± 14
Blood[a]	45 min	3 hr	846 ± 40	48 ± 6
Lung[a]	45 min	72 hr	849 ± 75	88 ± 24
Trachea[a]	3 hr	96 hr	925 ± 38	35 ± 17
Small intestine[b]	120 hr	–[e]	1,926 ± 521	311 ± 28
	192 hr[f]	–[e]	2,956 ± 159	335 ± 45

a) Migrating larvae were observed.
b) Adult worms were observed.
c) The first appearance of worms post inoculation.
d) The disappearance of worms post inoculation.
e) "–" means persistent infection.
f) Time of finding egg in feces.

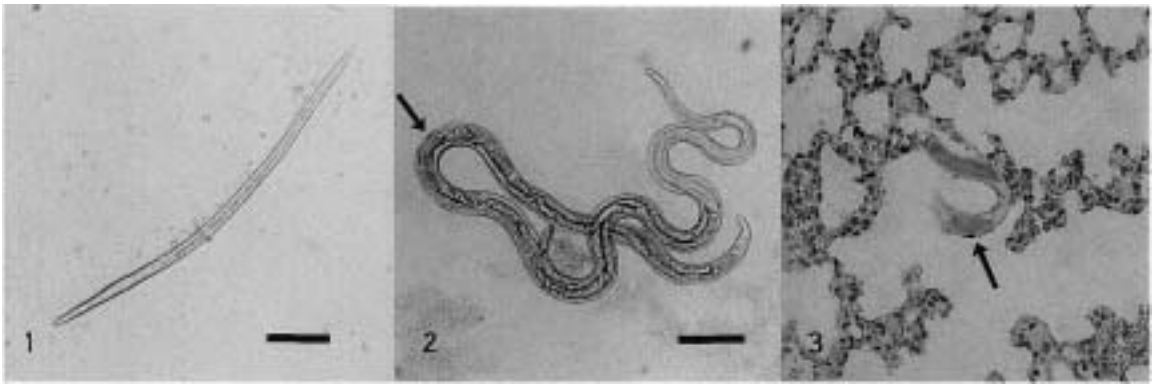

Fig. 1. A migrating larva of *Strongyloides venezuelensis* recovered from the lung of rat 3 hr post inoculation. Bar=100 μm.
Fig 2. A parasitic female of *Strongyloides venezuelensis* recovered from the intestine of rat 148 hr post inoculation. The indistinct eggs (arrow) at the primary stage of development were found in the uterus. Bar=100 μm.
Fig. 3. Lung of a rat 3 hr post inoculation with the infective larva of *Strongyloides venezuelensis*. A migrating larva (arrow) is observed in the alveolus near the interstitial layer. H & E stain. × 200.

Table 2. Chronologic development and fecundity of *Strongyloides venezuelensis* in Sprague-Dowley rats

Hours post inoculation	Length of body (μm) (Mean ± SD)	Eggs in the uterus or feces
120 hr	1,926 ± 521	indistinct contour of eggs in uterus
144 hr	2,278 ± 679	underdeveloped eggs in uterus
168 hr	2,420 ± 532	fully developed eggs in uterus
192 hr	2,956 ± 159	Eggs passed in feces

tled appearance with pink-reddish spots ranging from 1 to 3 mm in diameter, associated with congestive hepatomegaly and multifocal hemorrhagic necrotic lesions. Great numbers of eosinophils and MLS were also observed concurrently. A migrating larva observed in the lung 3 hr PI is shown in Fig. 3, traversing a hyperplastic interstitial layer.

DISCUSSION

The susceptibility of the host to the parasite is dependent

on the dosage of challenge and the immune status of the host. Susceptibility also determines the parasite load in the target internal organs [16, 21]. In the case of SVZ, the production/secretion of the adhesive substances by SVZ adult worms is a key step for the parasite to invade and establish itself in hostile environment created by the immune system of the host [14]. Khan *et al*. [11] stated that the Mongolian gerbil was unable to expel SVZ adult worms from the intestine for over 10 weeks, and Tsuji *et al*. [32] reported that the duration of fecal egg output and eggs per gram (EPG) were stable, and high EPG value continued for 450 days. Our observations in the previous report [4] are consistent with the published data [11, 32]. The Mongolian gerbil is an ideal animal model for maintenance of a certain species of parasites [4].

The intracorporal migration route of SVZ from the stomach to the liver and then through the blood stream to lung was verified by the data reported here. The key parameters included the body length of migrating worms, the time of their first appearance and disappearance of MLS in each target organ and the establishment of fecundity after oral inoculation [6]. Carter and Wilson [6] reported that SVZ failed to migrate from the skin to intestine in adult Wistar rats, the number of eggs in the uterus declined as the infection progressed and rats were idiosyncratic in their influence on parasite reproduction from the earliest time of sampling. In the Mongolian gerbil, the fecal egg output and fecundity of the worm remain relatively stable over a long period [4]. The EPG after oral inoculation has not been adequately described in comparison to the results obtained by subcutaneous or percutaneous inoculation. In this study, an oral inoculation of 1,000 SVZ L_3 directly into the stomach did not facilitate the recovery of AWS. It is noteworthy that the appearance time of AWS (120 hr PI) was actually delayed as comparing to that in rats subcutaneous inoculated (60 hr PI) with 2,000 L_3 [27]. The estimated prepatent period of 6 to 7 days PI is comparably shorter than that in the percutaneously inoculated rats using the same number of L_3 [26]. One previous report, however, concluded that suckling rats were effectively infected through mammalian transmission from inoculated nursing dams [18], whereas the others observed that an oral inoculation of SVZ to rats resulted in low infection rate [26]. Although in the last study, Takamure [27] intensively investigated the intracorporal distribution of MLS in subcutaneously inoculated rats from 24 to 96 hr PI, he did not mention any evidences about the liver as a migration route. As shown in our data in Table 1, the liver is considered as the site in the early-stage of migration within 20 min to 3 hr after oral inoculation. This suggestion coincides with the report by Petriello and Hardy [19]. They observed migrating larvae in the liver 24 and 48 hr PI with significant changes in liver-associated serum biochemical values from 12 to 96 hr PI, as well as in the heart and lung from 12 to 72 hr PI [19].

The present report failed to determine the migration route from the stomach to the liver and lung based on histopathological changes. The mild lesion formed during the intracorporal migration warrants additional studies emphasizing the comparisons of parasite strains along with immunocompetance studies in the experimental mammalian host. Our data demonstrated that the migration of MLS was not always associated with major pathological changes and that may explain why the Mongolian gerbil is such a good model for persistent infection [4, 32]. Sato and Toma [21] reported the use of *BALB*/c to study the course and intensity of SVZ infection as compared with *S. ratti* infection. The authors found mice to be much more susceptible to SVZ than *S. ratti*. The majority of worms inoculated were recovered from the lung and subsequently the small intestines and this migratory route was comparable to that of *S. stercoralis* in humans [17]. Different levels of infection, as assessed by fecal egg output, age, sex, and strain of mouse, were observed in mice infected with SVZ as well as in those infected with *S. ratti*.

The accumulation and significantly longer body of MLS in the lung are shown in Table 1. These observations are consistent with those of Taira *et al*. [26] and Takamure [27]. This observation reinforces the view that the concentration and growth of larvae in the lung may reflect the pathophysiological importance of the lung as a relay organ that permits the growth of parasite in the migration phase.

In conclusion, the appearance of larvae in various organs was remarkably faster than that has been previously reported. Our data provided a comprehensive basis for quantitative description of larval migration behavior after oral infection with SVZ and enhanced the value of the gerbil in the pathogenesis of this disease under natural and experimental situations.

ACKNOWLEDGMENT. This work was supported by a grant of Biosafety Research Institute(2002–13), Chonbuk National University, Korea.

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