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Evidence for the involvement of the optic nerve as a migration route for larvae in ocular toxocariasis of Mongolian gerbils

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Abstract

Although *Toxocara canis*, an important pathogen of ocular disease, tends to migrate to the eye, the precise migratory route has yet to be determined experimentally. Mongolian gerbils, *Meriones unguiculatus*, known as a useful animal model for human toxocariasis, were used to investigate the migration route toward the eyes. Infective larvae of *T. canis* were directly inoculated into the intracranial region. Haemorrhagic lesions or larvae were observed in 56.3% of cases. Histopathologically, a larva was observed in the optic nerve of gerbils 6 days after inoculation, and two larvae were found in the optic chiasma in the gerbils having a haemorrhage in the retina 9 days after inoculation. These results indicate that *T. canis* migrates from the brain to the eye through the optic nerve. Considering these data and previous studies showing that the ocular changes appear as early as 3 days of infection in the oral-administrated gerbils, there are two phases in the migration to the retina: a haematogenous early phase and an optic nerve route late phase.

Introduction

Toxocara canis and *T. cati* are ubiquitous gastrointestinal parasites in dogs and cats, respectively. Humans, especially young children, can be infected with these parasites following accidental ingestion of eggs containing infective-stage larvae. The migration of larvae in human tissues results in either ocular, visceral or covert disease syndromes. Ingested infective eggs of *T. canis* or *T. cati* hatch in the upper alimentary tract. After penetrating the intestinal wall, larvae disseminate through the systemic circulation, and subsequently spread to the muscles and central nervous system (CNS) (Glickman & Magnaval, 1993). Although the ocular form of this disease can cause a severe vision defect, there are few reports on the precise migratory route of larvae to the retina in orally infected hosts. Parke & Shaver (1996) suggested that larvae initially passed into the retina haematogenously. Maguire *et al.* (1990) speculated that larvae migrated to the retina through either the choroidal, central retinal, or ciliary vasculature. In contrast, Takayanagi *et al.* (1999) suspected direct migration of larvae from the brain to the retina along the optic nerve, since *Toxocara* larvae tend to accumulate in the brain. We demonstrated that some of the larvae that were inoculated directly into the brain migrated in the retina of gerbils, which are highly susceptible to *T. canis* (Takayanagi *et al.*, 1999) and *T. cati* infection (Akao *et al.*, 2000). The aims of the present study are therefore to show the evidence for the involvement of the optic nerve as an alternative migration route for larvae in ocular toxocariasis.

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Materials and methods

Animals

Seventeen 4-week-old male Mongolian gerbils, *Meriones unguiculatus*, were used in all experiments, and all were raised in our laboratory and kept under specific pathogen-free conditions in the Animal Centre of Tokyo Medical and Dental University.

Parasite collection and infection procedures

Adult females of *T. canis* were collected from puppies after administration of parbendazole (Sankyo Pharmaceutical Co., Japan). Eggs were obtained from the uteri of gravid females and cultured *in vitro* as described by Oshima (1961). Second-stage larvae were then collected aseptically and maintained in a culture medium until use. A narrow parietal region of skin in each gerbil was cut open under anesthesia with pentobarbital sodium (30 mg kg^{-1}, Pittoman-Moore, New Jersey, USA), and 300 second-stage larvae were directly inoculated intracranially through the cranial bone using a 23-gauge needle. Prior to inoculation, larvae were suspended in physiological saline after repeated washings with culture medium. The wounds were then closed with sutures. Initially, five gerbils were used for the longitudinal observation of the ocular changes after inoculation, and 12 gerbils were used for the histopathological investigation as well as for ocular observations.

After the operation, one of the gerbils died within 24 h. A small number of air bubbles may have been injected together with the *T. canis* larvae and might account for the death of this gerbil. Alternatively, the number of larvae we applied may have been excessive for the gerbil. In addition, six gerbils showed severe convulsion, with progressive emaciation and gerbils finally died from cachexia with neurological symptoms 2 weeks after inoculation. No visible changes in behaviour or appetite were noticed in the remaining gerbils. These experiments were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University, and have been properly carried out under the Guidelines for Animal Experimentation in the Tokyo Medical and Dental University.

Ocular observations

Under anesthesia with pentobarbital sodium, the ocular fundi of inoculated gerbils were carefully observed using an ophthalmoscope (Scalar VMS-170M, Abbe Science, Japan) after the pupils were dilated with tropicamide (Takayanagi *et al.*, 1999). The images of the fundi were transferred to a video monitor for viewing, and were recorded on videotape every 3 days from days 0 to 60.

Pathological examination and counting of larvae in the brain

The experimental design is shown in table 1. On days 6, 9 and 30, one of the gerbils with ocular changes in the second group was killed on each day for histopathological investigation. On days 12 and 15, gerbils which became weak due to cachexia with neurological symptoms, and died during the ocular observation under anesthesia, were also histopathologically examined. A total of five gerbils were examined and the eyeballs, together with the optic nerve and brain, were removed from the skull and immediately fixed in 2.5% glutaraldehyde and 4% formaldehyde in 0.15% phosphate buffer (pH 7.2). Serial sections embedded in JB-4 plastic resin were stained with haematoxylin and eosin, and examined as described by Takayanagi *et al.* (1999). One of the gerbil brains without ocular changes 6 days after inoculation was cut into small pieces and each piece was pressed between two glass slides so that the larvae could be counted under a microscope (Kondo, 1970).

Table 1. Experimental design for observing pathological changes in gerbils infected with *Toxocara canis* larvae.

	Mongolian gerbils	
	Group 1	Group 2
Number examined	5	12
Ocular observations	Every 3 days	Every 3 days
Histopathological observations	ND*	Day 6†, 9†, 12, 15, 30†
Counting larvae in the brain	ND*	Day 6

*Not done.
†On each day one of the gerbils with ophthalmoscopic changes was sacrificed.

Results

Haemorrhagic lesions and larvae

Either vitreous or choroidal haemorrhages were observed in the gerbils from 6 days after the intracranial inoculation. Choroidal and vitreous haemorrhages were observed at the peripheral region of the retina, and the optic papilla, respectively (fig. 1). Larvae were also observed simultaneously with the emergence of the haemorrhages. Haemorrhagic lesions or larvae were observed in nine of 16 gerbils (56.3%), two of which showed vitreous and choroidal haemorrhages, six had only choroidal haemorrhages, and one had larvae without haemorrhaging. Most of the ocular changes appeared on day 12. Abnormal ocular findings were observed in 12 of 32 eyeballs (37.5%) examined. A total number of 15 haemorrhagic legions occurred in eight gerbils, in which nine lesions were observed on day 12 for the first time. Once these ocular changes occurred, there were no new haemorrhages or larvae in the eye (table 2). Haemorrhagic legions were absorbed gradually within 1 month after they appeared. No granulomatous lesions were detected in any of the gerbils examined.

Pathological findings

Six of 12 gerbils of the second group were sacrificed for pathological studies or for counting larvae. No abscesses or haemorrhages were observed at the inoculation site. Microscopically, 329 larvae were counted in one gerbil brain 6 days after inoculation. Histopathological sections revealed a larva in the optic nerve of the gerbil 6 days after inoculation (fig. 2). After 9 days, two larvae were found in the optic chiasma plus a haemorrhagic legion and a larva in the retina (fig. 3). No inflammatory changes were observed around the larvae, although eosinophil infiltrations were scattered beneath the sheath of the optic nerve.

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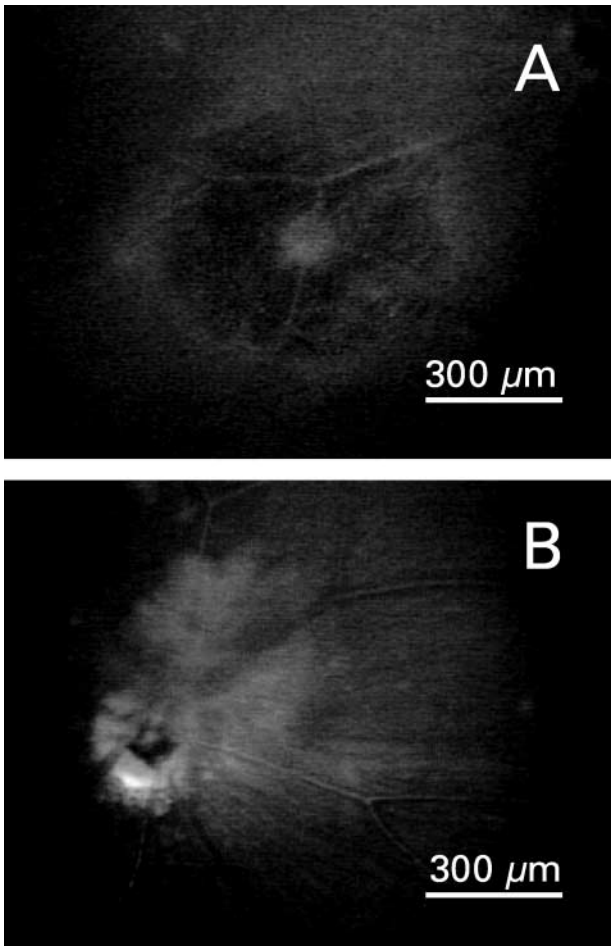

Fig. 1. Ocular fundi of the Mongolian gerbil following intracranial inoculation with *Toxocara canis* larvae: A, choroidal haemorrhage at peripheral region of retina. B, reddish vitreous haemorrhage around optic papilla.

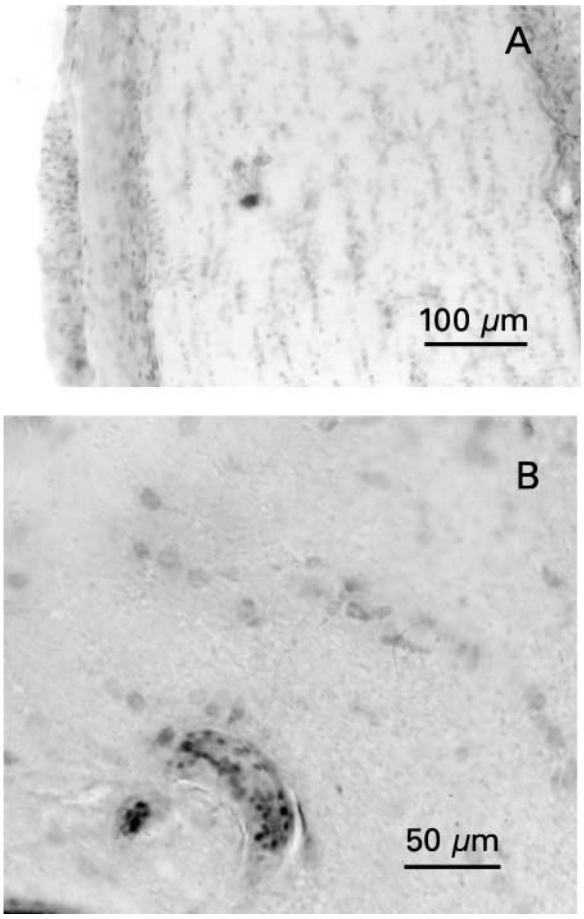

Fig. 2. Cross-section of the optic nerve of the Mongolian gerbil at day 6 following intracranial inoculation with *Toxocara canis* larvae: A, larvae migrating in the optic nerve. B, as A, but at a higher magnification.

Discussion

While there have been a number of clinical and experimental reports on ocular toxocariasis, it remains unclear just how larvae migrate to the retina. Maguire *et al.* (1990) suspected that larvae might enter the eye via the choroidal, central retinal and ciliary vasculature on the basis of their clinical cases. Shields (1984) stated that larvae reach the eye through ciliary vessels and the central retinal artery. Parke & Shaver (1996) suggested that most of the migration is initially haematogenous. On the other hand, Watzke *et al.* (1984) demonstrated that a larva appeared in the optic nerve of cynomolgus monkeys after intravitreal injection of live larvae, suggesting that *T. canis* larvae can make a retrograde migration from the eye to the central nervous system.

Table 2. Ophthalmoscopic changes in Mongolian gerbils after inoculation with *Toxocara canis* larvae intracranially. Approximately 300 larvae were injected into the brain with a 23-guage needle, and ocular changes were observed every 3 days from day 0 to day 60.

Haemorrhagic lesions with larva	Haemorrhagic lesions without larva	No haemorrhagic lesions with larva	No ophthalmological changes	Total
Day 6* (1)†	Day 6 (1)			
Day 12 (3)	Day 9 (1)	Day 15 (1)	(7)	(16)
Day 18 (1)	Day 12 (1)			

*Days after inoculation when the lesions were observed for the first time.
†The number of gerbils observed is shown in parentheses.

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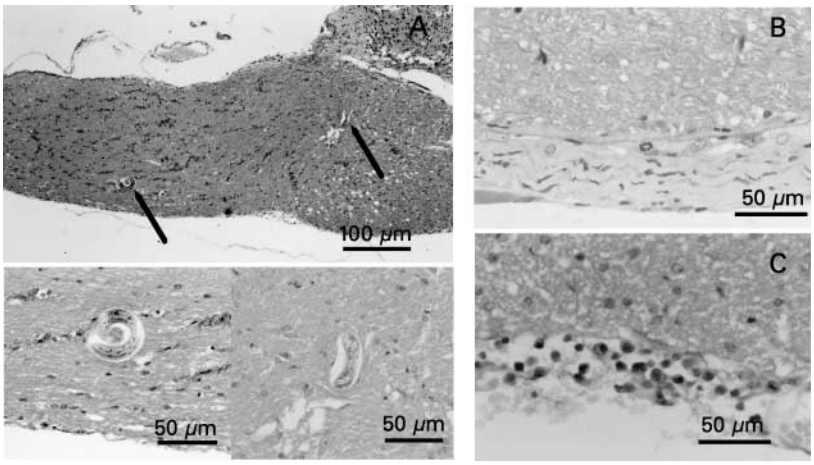

Fig. 3. Cross-sections of the optic chiasma of the Mongolian gerbil at day 9 following intracranial inoculation with *Toxocara canis* larvae: A, two migrating larvae, in the optic chiasma, and higher magnification. B, eosinophil infiltration and C, mononuclear cell infiltration beneath the optic nerve sheath.

In the present study, *T. canis* larvae were shown to migrate from the brain to the retina of gerbils through the optic nerve. Choroidal and vitreous haemorrhages were observed in the retina of gerbils inoculated with larvae, although no vascular changes were noted. Previously, Takayanagi *et al.* (1999) reported that ocular changes occurred 3 days after oral administration, which was clearly earlier than those in the present study, indicating that larvae move into the retina not only along the optic nerve but also via a haematogenous route. Larvae may migrate directly through the arteries from the internal carotid artery to the ophthalmic, retinal central or ciliary arteries. Takayanagi *et al.* (1999) showed that choroidal haemorrhages with or without larvae re-emerged after oral infections of gerbils. The present data, therefore, suggest that a re-emerged lesion could be attributed to a larva arriving late. In addition, the incidence of ocular changes was low (56.3%) compared with the oral inoculation (95%) (Takayanagi *et al.*, 1999). These data also strongly indicate that following oral inoculation, larvae accumulate in the eye both haematogenously and neurotropically in gerbils.

There have been several studies on experimental ocular toxocariasis. Among these, mice (Kunishige, 1964; Olson, 1976; Ghafoor *et al.*, 1984), rabbits (Kunishige, 1964), guinea pigs (Miyamoto, 1972; John *et al.*, 1983) and monkeys (Luxenberg, 1979; Watzke *et al.*, 1984) were evaluated as animal models for this disease. However, none of these animal models exhibited a high incidence of ocular lesions following a single oral inoculation. Additionally, there are no reports on the migration route of the larvae involved in an infection of the eye. In the present study, when larvae were injected into the iliac vein of gerbils to determine whether larvae had migrated through the blood vessels, no ocular change was observed (data not shown).

Larvae injected directly into the brain of NIH mice were capable of migrating into the viscera and musculature (Abo-Shehada & Harbert, 1984). In contrast, we found that larvae that had migrated to the brain remained there, although some migrated to the eyes. These data suggested that larvae have an affinity for the CNS and eyes in gerbils. However, further studies are needed to clarify the migratory route of larvae following arrival in the brain.

Histopathologically, we found a larva in the optic nerve and optic chiasma. No pathological changes were observed around the larva, neither in the optic nerve nor in the brain, including the optic chiasma. Interestingly, eosinophil infiltrations were present in the optic nerve sheath that were unrelated to the larva; although eosinophilic granulomata are frequently found in human ocular toxocariasis (Irvine & Irvine, 1959; Duguid, 1961; Harris, 1961; Rey, 1962). No granulomatous lesions were detected in any of the gerbils in the present study. As Takayanagi *et al.* (1999) suspected, motile larvae would not be able to induce a local immune response as long as they were alive. However, the present study suggests that cytokines, including an eosinophil chemotactic factor, might be produced in the optic nerve, but further studies are needed to confirm this.

Kira *et al.* (1997) reported that infiltrations of eosinophils in the CNS, especially in the spinal cord, associated with atopic diseases should be referred to as atopic myelitis. Although the aetiology of this disease is unknown, Kira *et al.* (1997) assumed that helminth infections might be associated with atopic myelitis. In this respect, the gerbil might also be a suitable animal model for neurological toxocariasis.

In the present study, some gerbils became weak and died due to cachexia with neurological symptoms and severe convulsion. Neither an abscess nor haemorrhaging in the brain was found macroscopically in these cases, suggesting that the symptoms were associated with a larval infection, either directly or indirectly. Gerbils orally inoculated with larvae showed neurological symptoms by 2 months after inoculation. The brains of these gerbils showed no inflammatory changes, but degenerative alterations in the axon of neurons in the cerebellum have previously been reported (Tomoda *et al.*, 2000).

The route of infection in ocular toxocariasis has been unclear, with the debate focusing on routes that are either

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haematogenous or neurotropic. Sero-negative cases are not unknown in human cases (Glickman & Magnaval, 1993). Furthermore, optic neuritis caused by *T. canis* has been diagnosed in humans (Komiyama *et al.*, 1995). Considering the previous reports and the present findings, we suspect that larvae sequestered in the brain for a long period of time might begin to migrate to the eyes through the optic nerve in response to changes in the host's physiological status under some form of stimulation, such as hormone changes, aging, or an immunodeficiency.

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