CLINICAL AND PATHOLOGICAL FEATURES OF EXPERIMENTAL ACANTHAMOEBA KERATITIS IN RABBITS

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Abstract: Experimental *Acanthamoeba* keratitis was induced in 24 female Dutch rabbits to examine the clinical effects of infection in the eye and to study the usefulness of a new histopathological technique for evaluating rabbit models of this infection. One eye of each animal in group A or B received an instillation of 1.3x10^4 (group A) or 1.3x10^5 (group B) amoeba cysts/eye; phosphate-buffered saline (PBS) solution was instilled into the contralateral eye as the control; animals in groups C and D received intrastromal injections of 5.0x10^3 (group C) or 1.5x10^4 (group D) cysts/eye in one eye and injection of an equal volume of PBS in the contralateral eye. Animals were observed daily for 5 to 84 days. Two rabbits in group D were killed on day 5 and enucleated eyes were embedded in paraffin and stained with hematoxylin-eosin or iodine-potassium iodide. In groups A and B, clinical signs of corneal injury disappeared by 4 hours after inoculation and signs of infection disappeared by day 2. In contrast, all eyes that had been injected with *Acanthamoeba* (groups C and D) developed severe keratitis, including keratoneuritis and corneal ulcer, followed by neovascularization or corneal perforation. Histologic examination showed infiltrates of leukocytes, lymphocytes, eosinophils, plasmacytes and spindle-shaped cells. The most extensive cell infiltration, and also exocytosis, liquefaction degeneration and intrastromal trophozoites, were seen in the limbic conjunctiva and palpebral conjunctiva. In addition, there was evidence of migration of inflammatory cells to the ciliary body and intravitreal space. This study showed that injection of *Acanthamoeba* into corneal stroma causes severe infection of the cornea and other eye tissues and that iodine-potassium iodide staining of paraffin embedded specimens is useful to detect *Acanthamoeba* trophozoites and cysts.

Key words: *Acanthamoeba* keratitis, Rabbit, Corneal ulcer, Keratoneuritis, Pathological feature, Iodine-potassium iodide staining

INTRODUCTION

The persistent and severe *Acanthamoeba* keratitis occurs occasionally in contact lens wearers and swimmers. Clinical findings in such cases include granular superficial corneal opacity, scleritis, discoid ulcer, pseudodendritis, stromatitis with ring infiltration. Thinning of cornea leading to corneal perforation may also occur when there is delay in accurate diagnosis and effective treatment of this condition (Wright et al., 1985). However, accurate diagnosis and effective treatment for amoebic keratitis is difficult because amoebae are encysted in infected tissues (Osato et al., 1991).

Rabbit models of *Acanthamoeba* keratitis have been used to study more effective techniques for diagnosis and treatment of this condition. The first rabbit model was reported by Font and colleagues, who demonstrated that stromal keratitis and the corneal ulceration can be induced by the intrastromal injection of *A. polyphaga* after subconjunctival administration of corticosteroid for 4 days (Font et al., 1981). They reported that corneas were culture-positive at intervals, and encysted amoebas have been observed (Font et al., 1981). Another rabbit model was reported by Lin et al. (1989), who investigated the effectiveness of steroid treatment of *Acanthamoeba* keratitis. A rat model of *Acanthamoeba* keratitis was reported by Larkin and Easty (1990). They induced *Acanthamoeba* keratitis in Wister rats by intrastromal inoculation of *A. polyphaga* cysts and reported that on histopathologic examination, sections of corneas showed liquefactive stromal necrosis and *Acanthamoeba*...
cysts in deep stroma (Larkin and Easty, 1990).

We undertook the present study to investigate the clinical and pathological features of several models of Acanthamoeba ocular infection, specifically the effects on tissues other than the cornea, such as the conjunctiva, ciliary body, and vitreous; and secondarily, to determine whether iodine-potassium iodide staining of paraffin-embedded sections might be useful in studying rabbit models of Acanthamoeba infection.

MATERIALS AND METHODS

Acanthamoeba isolated from a patient with Acanthamoeba keratitis were grown at 25°C on 1% agar plate with an overlay of heat-killed Escherichia coli as a food source. On the basis of differences in the size and morphologic features of the cysts, the protozoan was identified as Acanthamoeba polyphaga (group 2) (Fig. 1) (Font et al., 1981; Visvesvara, 1991).

Acanthamoeba cysts were instilled or injected into the eyes of 24 female Dutch rabbits that weighed 2.6 (standard deviation: 0.3) kg each. The rabbits were divided into four groups. Rabbits groups A (n=4) and B (n=4) received 9 linear latticed abrasions on the corneal epithelial layer, extending to the upper stromal layer. Then a suspension of cysts (1.3 × 10^4 amoebas/eye for group A or 1.3 × 10^5 amoebas/eye for group B) was instilled into one eye. Contra-lateral eyes of rabbits in groups A and B were instilled with an equivalent volume of phosphate buffered saline (PBS) solution. Rabbits in groups C (n=8) and D (n=8) received an injection of 5.0 × 10^3 cysts/kg body weight (b.w.) (20 µl) (group C) or 1.5 × 10^4 cysts/kg b.w. (50 µl) (group D); the injection was made intrastromally at the 2 o’clock position on the cornea, about 2.5 mm from the limbus. Contra-lateral eyes of rabbits in group C or D were injected with an equal volume (20 µl or 50 µl, respectively) of PBS.

Slit-lamp examinations of the anterior segments of all eyes of all rabbits were performed hourly for the first 5 hr and every day for up to 84 days after the inoculation. Two rabbits in group D were killed and enucleated by intravenous injection of sodium pentobarbital on day 5, and all the other rabbits were sacrificed on day 84 after the inoculation. The enucleated eyes of rabbits were embedded in paraffin and sectioned at 3-5 µm. Sections were then stained with hematoxylin-eosin (H-E) or iodine-potassium-iodide and examined by light microscopy.

RESULTS

1. Clinical features of Acanthamoeba instillation

(1) Group A and B

By 4 hrs after injury and instillation of Acanthamoeba in eyes in groups A and B, all signs of corneal injury had disappeared. On day 1 after the inoculation, hyperaemia and a few dot haemorrhage were seen in the conjunctivas of eyes that had received instillation of Acanthamoeba, but these manifestations were disappeared on day 2. Daily examination of eyes in groups A and B for the remainder of the 12-week study showed on signs of corneal disorder and no recurrence of conjunctivitis. There was no apparent difference between group A and B eyes in the clinical response to corneal injury and instillation of Acanthamoeba cysts.

(2) Group C and D

In inoculated eyes in groups C and D, corneal opacity occurred at the site of inoculation within minutes after inoculation and disappeared within 5 hrs after the inoculation. Simultaneously, hyperaemia and dot haemorrhages become evident in the palpebral conjunctiva. Cellular infiltration in an inoculated site of the cornea, keratoneuritis and corneal ulcer were seen in the area of inoculation on day 2 after inoculation in eyes in group C and on day 1 after inoculation in eyes in group D (Fig. 2). In rabbits in group C, the corneal lesions gradually increased in area until day 5 after the inoculation. On day 6, pannus appeared and corneal infiltration start to disappear. Small cystic lesions were found in the cornea on day 8, and these enlarged until day 14. The corneal neovascularization was noted at the limbic conjunctiva on day 14 and developed into a corneal ulcer. After day, 28, the cystic lesions in the corneas began to decreased in size and replaced by nubecula. By day 49, only small area of pannus and nubecula remained on the corneas of rabbits in group C (Fig. 3), and there were no signs of recurrent keratitis during the remainder of the 12-week period after instillation of Acanthamoeba in the eyes of rabbits in group C.

In rabbits group D, the corneal lesion appeared granular and turbid on day 2 after inoculation and had enlarged to involve half of the corneal surface. On day 4, a portion of the corneas in 2 eyes (2 rabbits) in group D was noticeably thinner (Fig. 4), and on day 7 perforation was noted at these site.

The significant clinical difference between rabbits in group C and those in group D was that corneal lesions in group C showed spontaneous resolution, while those in group D increased, resulting in corneal perforation.

2. Histological features of Acanthamoeba keratitis

In the 2 eyes of rabbits in group D that were killed on day 5 after inoculation of Acanthamoeba, histological ex-
Figure 1 Iodine-potassium iodide stained *Acanthamoeba* cysts. Bar: 10 μm

Figure 2 Biomicroscopic appearance of a rabbit cornea in group D, 1 day after inoculation of *Acanthamoeba*. A corneal ulcer, stromatitis and keratoneurtis have developed.
Figure 3 Biomicroscopic appearance of a rabbit cornea in group C at 7 week after inoculation of *Acanthamoeba*. The corneal ulcer has disappeared, and pannus and nubecula are seen.

Figure 4 Biomicroscopic appearance of a rabbit cornea in group D on day 4 after inoculation of *Acanthamoeba*. Thinning of the cornea is evident.
Figure 5 Histologic appearance of a rabbit cornea 5 days after inoculation with \textit{Acanthamoeba}. The corneal stroma shows infiltration of cells, including polymorphonuclear leukocytes (P), lymphocytes (L), eosinophils (E), plasmacytes (Q), spindle-shaped cells (S) and trophozoites (T), surrounded by liquefaction products. Hematoxylin-eosin staining. Bar: 10 μm.

Figure 6 Histologic appearance of a rabbit cornea 5 days after inoculation with \textit{Acanthamoeba}. Iodine-potassium iodide staining of the paraffin embedded section differentiates cysts from trophozoites (T). Bar: 20 μm.

Figure 7 Histologic appearance of rabbit limbic conjunctiva 5 days after inoculation with \textit{Acanthamoeba}. Infiltrates of lymphocytes (L), polymorphonuclear leukocytes (P) and plasmacytes (Q) and several trophozoites (T) are visible. Lymphocytes and plasmacytes may adhere to the walls of dilated blood vessels (D). Bar: 50 μm.
Figure 8 Histologic appearance of rabbit palpebral conjunctiva 5 days after inoculation of *Acanthamoeba*. Infiltration of inflammatory cells (P) into the palpebral conjunctiva, exocytosis (e) and migration of trophozoites (T), and dilated blood-vessels (D) are visible. Bar: 30 μm

Figure 9 Histologic appearance of rabbit ciliary body 5 days after inoculation of *Acanthamoeba*. Polymorphonuclear leukocytic (P) infiltration and multinucleated giant cells (M) are visible in the ciliary body and intravitreal space. Bar: 50 μm
amination showed remarkable cell infiltration in the corneal stroma, including polymorphonuclear leukocytes, lymphocytes, eosinophils, plasmacytes, spindle-shaped cells and trophozoites, surrounded by an area of liquefactive degeneration (Fig. 5).

On iodine-potassium iodide stained paraffin embedded sections of these eyes, it was possible to differentiate cysts from trophozoites (Fig. 6). Changes in the limbus conjunctiva 5 days after inoculation of *Acanthamoeba* cysts were infiltrations of lymphocytes, polymorphonuclear or eosinophilic leukocytes, plasmacytes and scattered trophozoites. Dilatation of intravascular spaces and neovascularization were also evident in the limbus conjunctiva of rabbits in group D 5 days after inoculation of *Acanthamoeba* cysts (Fig. 7). In the palpebral conjunctiva, H-E staining showed in filtrations of inflammatory cells and trophozoites and exocytosis in the epithelial layer (Fig. 8). H-E staining showed polymorphonuclear leukocyte infiltrations in the ciliary body and the anterior vitreous, trophozoites in the ciliary body, and multinucleated giant cells in the intravitreal space (Fig. 9).

**DISCUSSION**

*Acanthamoeba* has a life cycle of trophozoite and dormant cyst stages.

*Acanthamoeba* keratitis was first described clinically more than 30 years ago, however, starting in 1985 the number of cases of amoebic keratitis reported has grown each year (Warhurst and Mann, 1988; Wright and Buckley, 1988; Wright et al., 1985).

The pathogenesis of *Acanthamoeba* keratitis has been studied using a variety of experimental models. Culbertson *et al.* (1959) examined the results of intravenous injection or intranasal inoculation of *Acanthamoeba* organisms into mice and monkeys, and others have developed experimental models of amoebic keratitis in a variety of species, including rabbits, hamsters, swine and mice (Font *et al.*, 1981; Larkin and Easty, 1990; Klink *et al.*, 1994; Alizaeh *et al.*, 1995; Ruddell and Easty, 1995; John *et al.*, 1991). Larkin and Easty (1990) reported on the histological appearance of the cornea in eyes with *Acanthamoeba* keratitis. However, there is little information about the effects of *Acanthamoeba* infection on other eye tissues, including the conjunctiva, iris, intravitreal space and ciliary body.

In the present study, we created the first reported reproducible model of *Acanthamoeba* keratitis in rabbit eyes and documented changes in the cornea, limbic or palpebral conjunctiva, ciliary body and anterior vitreous after intrastromal injection of a suspension of *Acanthamoeba* cysts. Our results show that intra-corneal injection of *Acanthamoeba* cysts leads to the development of small cystic lesions in the cornea that can be observed clinically by slit-lamp examination. In addition, paraffin-embedded sections of rabbit eyes obtained 5 days after inoculation showed the presence of inflammatory cell infiltrates and *Acanthamoeba* trophozoites in other parts of the eye, such as limbic conjunctivae, palpebral conjunctivae, ciliary body, as well as the intrastromal spaces in the cornea.

Differentiating between the cyst and the trophozoite stages of the *Acanthamoeba* life cycle is helpful because almost all clinical specimen were found as the trophozoite stages of the *Acanthamoeba*. In our study, corneal smears and histologic sections stained with iodine-potassium iodide were useful for differentiating cysts from trophozoites. As a result, detailed studies of the *Acanthamoeba* life cycle in keratitis using anti-amoebic monoclonal antibodies are planned for the near future.

In patients, the course of *Acanthamoeba* keratitis is often prolonged in spite of intense treatment. Corneal curettage is the most effective treatment, especially when *Acanthamoeba* infection is confined to the cornea, because most anti-amoebic drugs are not very effective against encysted *Acanthamoeba*. The results of our study suggest that migration of *Acanthamoeba* trophozoites from the cornea to other parts of the eye, such as the palpebral or limbic conjunctiva, ciliary body and anterior vitreous, may help explain the difficulty of treating severe *Acanthamoeba* keratitis and the occurrence of complications such as endophthalmitis or panophthalmitis.

**REFERENCES**