CULTIVATION OF GIARDIA DUODENALIS IN MONGOLIAN GERBILS

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Abstract. The Mongolian gerbil (Meriones unguiculatus) is susceptible to infection with Giardia duodenalis trophozoites. Each animal was orally infected with 0.5 ml Diamond’s TYIS-33 culture medium containing $10^6$ trophozoites. Cysts were then collected and concentrated by sucrose gradient centrifugation. G. duodenalis cysts were first observed in feces on day 5 post-infection. The characteristic of G. duodenalis infection in gerbils was intermittent cyst release. The range in the number of cysts released per gerbil for a 4-hour collection period was 0-1.5 $\times 10^3$.

INTRODUCTION

Giardia duodenalis (syn. Giardia intestinalis, Giardia lamblia) is a flagellated unicellular eukaryotic microorganism that commonly causes diarrheal disease worldwide. Experimental infections of Giardia have been studied in a variety of animals. Mice (Vinayak et al, 1979; Hill et al, 1983), rats (Anand et al, 1980; Craft, 1982; Kanwar et al, 1986) and gerbils (Belosevic et al, 1983) have been most commonly used in the development of laboratory models; sometimes, cats (Kirkpatrick and Green, 1985), dogs (Hewlett et al, 1982), rabbits (Schleinitz et al, 1983), and sheep (Olson et al, 1995) have also been utilized. The Mongolian gerbil (Meriones unguiculatus) is widely regarded as the best experimental host of G. duodenalis infections and offers a much better alternative to mice as a laboratory model. In this study, we aimed to determine whether in vitro-grown trophozoites were infective to gerbils, and to describe the G. duodenalis cyst collection technique for viability, inactivation, or elimination study.

MATERIALS AND METHODS

Experimental animals

Male Mongolian gerbils (Meriones unguiculatus) 6-10 weeks old, weighing 40-50 g, were used in this experiment. The animals were purchased from the Laboratory Animal Unit, Faculty of Medicine, Khon Kaen University, Thailand. On arrival at the facility, the gerbils were placed in filter-top cages (one gerbil per cage) with food and water provided ad libitum.

Gerbils were treated for 3 consecutive days with a solution (20 mg per gerbil per day) of metronidazole (Flagyl®; Rhone-Poulenc, Montreal, Quebec, Canada), administered by gavage before experimental infection. This treatment ensured that the gerbils were free from all previous infections of the small intestine, as demonstrated by 3 consecutive examinations of feces of gerbils chosen at random.

Preparation of G. duodenalis trophozoites

Trophozoites of G. duodenalis were routinely maintained in vitro in Diamond’s TYIS-33 culture medium at the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Thailand. Actively growing G. duodenalis trophozoites (48-to-96 hour-old culture) were incubated on ice for 20 minutes to detach the parasite from the walls of the screw-cap 16 by 125-mm culture tube. The organisms were then sedimented by centrifugation at 2,500 rpm for 10 minutes at 4°C, counted, and resuspended in 0.5 ml culture medium to contain $10^6$ trophozoites.

Inoculation of G. duodenalis trophozoites

Gerbils were inoculated orally through a feeding tube with 0.5 ml of culture medium containing $10^6$ G. duodenalis trophozoites.

G. duodenalis cyst collection

Cysts released in 4 hours’ fecal collection from the gerbils were collected in 12 by 75-mm plastic tubes during days 1-14 post-infection. The cysts were first observed in feces on day 5 post-infection. The characteristic of G. duodenalis infection in gerbils was intermittent cyst release. The range in the number of cysts released per gerbil for a 4-hour collection period was 0-1.5 $\times 10^3$.

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water-sucrose interface were carefully removed with a Pasteur pipette, washed by resuspension in 4 ml of normal saline, and sedimented by centrifugation at 600g for 10 minutes. The supernatant was removed, the cysts resuspended in phosphate-buffered saline and counted in a hemacytometer chamber. Finally, the cysts were stored at 4°C in phosphate-buffered saline and 0.01% Tween 20 with antibiotic cocktail (100 U penicillin and 0.1 mg/ml streptomycin) to inhibit bacteria growth until used.

RESULTS

Cysts were first observed in feces on day 5 post-infection (Fig 1). The characteristic of G. duodenalis infection in gerbils was intermittent cyst release, and the number of cysts released per gerbil for a 4-hour collection period ranged from 0 to 1.5 × 10³.

DISCUSSION

This study demonstrated that Mongolian gerbils are susceptible to infection with G. duodenalis trophozoites. Intermittent cyst release was characteristic of G. duodenalis infection in gerbils. Our result is similar to previous studies with Mongolian gerbils (Belosevic et al, 1983; Bouza et al, 2000) and mongrel dogs (Hewlett et al, 1982). However, the number of cysts excreted by these gerbils was lower than recorded by Belosevic et al (1983). The difference is probably due to the fact that Belosevic et al (1983), infected orally via a stomach tube (intragastric gavage), while we infected orally via a feeding tube (intraesophagus gavage). Experimental infections of mice by a single trophozoite of G. muris (de Carneri et al, 1977), dogs (Hewlett et al, 1982), domestic cats (Kirkpatrick and Green, 1985) and Mongolian gerbils (Belosevic et al, 1983), by cultured trophozoites from humans, have been reported. Although the trophozoite is not thought to be the usual form of transmission, it is important to recognize that infection by this form of the parasite is possible and that gastric acidity does not seem to affect trophozoite infectivity. Mongolian gerbils appear to be an excellent model for the study of G. duodenalis because (1) they can be infected with isolates from a variety of hosts; (2) adult animals can be infected with either cysts or trophozoites cultured in vitro; (3) their prepatent period and pathogenesis are similar to those of the original hosts; (4) they are capable of maintaining strains of G. duodenalis in the laboratory by serial passage; (5) they are prolific and easy to breed; and (6) they can be infected with a low infectious dose (ID₅₀, 5-15 cysts) (Stachan and Kunst, 1983; Hibler et al, 1987; Thompson, 1999). In this study, we also describe the collection technique for G. duodenalis cysts using sucrose gradient centrifugation (Roberts-Thomson et al, 1976). We found the sucrose gradient centrifugation technique suitable for viability, inactivation, or elimination studies.

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