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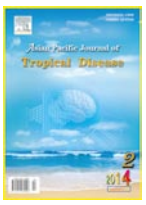

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Brain cystogenesis capacity of *Toxoplasma gondii*, avirulent Tehran strain in mice

Mehrzad Saraei[1,2], Yosef Ghaderi[1], Tahereh Mosavi[1], Mojtaba Shahnazi[1], Hossein Keshavarz[3], Saeedeh Shojaee[3*]

[1]*Department of Medical Parasitology and Mycology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran*

[2]*Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran*

[3]*Department of Medical Parasitology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran*

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ABSTRACT

Objective: To investigate the brain cystogenesis capacity of Tehran strain of *Toxoplasma gondii* (*T. gondii*) that had been isolated from a patient with lymphadenitis in 1973.

Methods: A volume of 0.5 mL mice brain suspension containing 20 tissue cysts of Tehran strain of *T. gondii* was inoculated intraperitoneally to each of 25 male BALB/c mice. The number of brain cysts was counted in unstained squash-smears for 10 mice during weeks 7-9 and for 15 mice during weeks 13-14 post-infection. Nonparametric test of Mann-Whitney was used to demonstrate means differences.

Results: There was a significant difference in the means for the number of brain cysts between weeks 7-9 (228.3±144.8) and weeks 13-14 (1 239.8±429.3) post-infection ($P<0.05$). The minimum and the maximum of cysts were 70 and 1 531 during weeks 7-9 post-infection, and 12 and 5 170 during weeks 13-14 post-infection, respectively. The mean number of brain cysts in the right cerebral hemisphere was insignificantly higher than that of the left cerebral hemisphere. Furthermore, the number of cysts counted in the right or the left hemispheres was significantly higher than those enumerated for cerebellum+brain stem altogether.

Conclusions: It is concluded that the brain cystogenesis capacity of *T. gondii*, Tehran strain shows enormous variation in mice regarding the duration of infection. In addition, the cystogenesis observed in cerebellum+brain stem is lower than the right and left cerebral hemispheres.

1. Introduction

Toxoplasma gondii (*T. gondii*) is one of the most common protozoan parasites in humans and warm-blooded animals worldwide, so that, at least 20% of world populations are seropositive for this infection in most of developing[1-3] and developed countries[4-6].

Tissue cystogenesis is a part of developmental process of *T. gondii* occurring in both definitive and intermediate hosts. Tissue cysts are formed in many organs of the hosts, however, the frequency and distribution of cysts are partly controlled by the host and the strain of *T. gondii* involved[7]. In rats, higher number of tissue cysts is found in brain rather than in other organs[8], and therefore, the brain is considered as a selective organ for the *in vivo* cystogenesis of this parasite[9,10].

It has been shown that the tissue cysts form as early as six days post-infection in mice brain. They grow regularly and their size is stopped within less than 4 months. Young brain cysts may be measured as small as 5 μm in diameter containing only 2 bradyzoites, and older cysts reach up to 50-70 μm in diameter containing hundreds of bradyzoites[7]. The size of cysts may exceed 100 μm, as described in a report by Hooshyar *et al.* in which a cyst of about 125 μm was observed in the brain of a mouse, experimentally infected with local isolates of *T. gondii*[11].

Brain cystogenesis capacity as a biological characteristic shows significant diversities among avirulent strains of *T. gondii*[12]. Awareness of this capability of *T. gondii* strains will be helpful in using this parasite in chronic infections studies in mice. The Tehran strain of *T. gondii* is an

*Corresponding author: Saeedeh Shojaee, Department of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
Tel/Fax: +982188951392
E-mail: s_shojaee@tums.ac.ir
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avirulent strain isolated by Ghorbani *et al.* from a patient with lymphadenitis in Tehran, the capital of Iran, in 1973[13]. Since the initial isolation, it is maintained at the Department of Parasitology, Tehran University of Medical Sciences through intraperitoneal passages of brain homogenate containing the tissue cysts of this strain in mice. This strain has been used in a molecular study[14], however, this is the first report concerning the cystogenesis capacity of this strain in the brain of mice.

2. Materials and methods

2.1. Experiment

Brain suspension in saline was prepared from the mice infected with tissue cysts of *T. gondii*, Tehran strain three months earlier. A volume of 0.5 mL brain suspension containing 20 tissue cysts of Tehran strain was inoculated intraperitoneally to each of 25 male BALB/c mice (animals were seronegative for anti-*T. gondii* antibodies by Sabin-Feldman dye test)[15]. Mice were purchased from Razi Vaccine and Serum Research Institute and housed in plastic cages with food and water available *ad libitum*. Ten mice at weeks 7-9 and fifteen mice at weeks 13-14 were deeply anesthetized by intraperitoneal injection of ketamine (150 mg/kg) and xylazine (15 mg/kg), followed by removal of their brains from the skulls. Each brain was divided into 3 sections including, right cerebral hemisphere, left cerebral hemisphere, and the cerebellum+brain stem. Unstained squash-smears were prepared from the whole brains and the numbers of tissue cysts were counted at two magnifications of 100× and 400× using light microscopy.

2.2 Ethical considerations

The study protocol was approved by the Ethical Review Board of Qazvin University of Medical Sciences, Qazvin, Iran.

2.3. Statistical analysis

The data were analyzed by SPSS version 13. Nonparametric test of Mann-Whitney was used to demonstrate means differences. A *P*-value less than 0.05 was considered as significant.

3. Results

The number of tissue cysts in the brain of mice showed remarkable variations. The minimum and maximum number of cysts were 70 and 1531 at weeks 7-9 post-infection, and 12 and 5170 at weeks 13-14 post-infection, respectively. The frequency distribution of brain cysts in mice with experimental infection to *T. gondii*, Tehran strain is shown in Table 1.

Table 1

Frequency of tissue cysts in brain of 25 BALB/c mice inoculated intraperitoneally with 20 tissue cysts of *T. gondii*, Tehran strain at weeks 7-9 and 13-14 post-infection.

Weeks 7-9 (Group 1)		Weeks 13-14 (Group 2)			
Mice number	Number of tissue cysts	Mice number	Number of tissue cysts	Mice number	Number of tissue cysts
1	1531	1	112	11	476
2	117	2	208	12	153
3	72	3	312	13	59
4	87	4	302	14	12
5	89	5	2749	15	5170
6	70	6	549		
7	86	7	660		
8	95	8	2992		
9	55	9	4089		
10	81	10	754		

There was a significant difference in the means observed for the number of brain cysts between Group 1 and 2 ($P<0.05$). The mean±SEM numbers of brain cysts in Group 1 and 2 were 228.3±144.8 and 1239.8±429.3, respectively.

Overall, the mean number of brain cysts in the right cerebral hemisphere was higher than the left cerebral hemisphere; however the difference was not significant, statistically. On the other hand, the number of tissue cysts in the cerebellum+brain stem was significantly lower than that observed in the right and left cerebral hemispheres ($P<0.05$) (Table 2).

Table 2

Frequency of tissue cysts in the right cerebral hemisphere, left cerebral hemisphere, and cerebellum+brain stem of 25 BALB/c mice inoculated intraperitoneally with 20 tissue cysts of *T. gondii* Tehran strain at weeks 7-9 and 13-14 post-infection (mean±SEM).

Mice brain	Weeks 7-9 (Group 1)	Weeks 13-14 (Group 2)
Right cerebral hemisphere	78.30±40.79	625.60±221.34
left cerebral hemisphere	123.10±88.73	427.40±144.83
cerebellum+brain stem	26.90±15.37	186.80±68.59
Total	228.30±144.80	1239.80±429.30

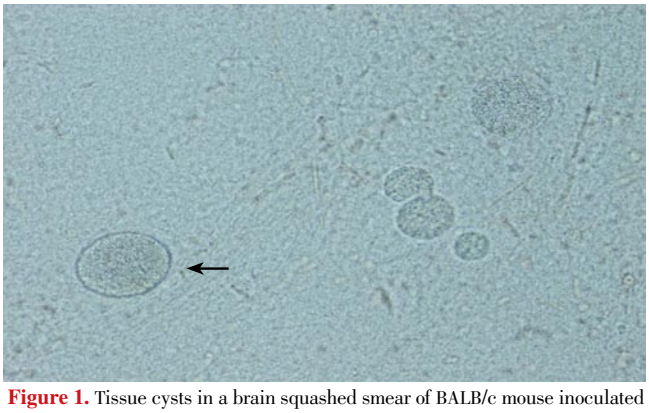

Figure 1. Tissue cysts in a brain squashed smear of BALB/c mouse inoculated intraperitoneally with tissue cysts of *T. gondii* at week 14 post-infection. Note the larger size (3-4 folds) of brain cysts at magnification 200×.

The tissue cysts observed microscopically showed different dimensions; so that a difference of 3–4 folds in the diameter of cysts was obvious at weeks 13–14 post–infection (Figure 1).

4. Discussion

In the present study, the tissue cysts were observed in the brain of all mice experimentally infected with *T. gondii*, Tehran strain, however, there was a significant difference in the brain cystogenesis capacity of this strain in mice; despite the similarity in the inoculum size and the number of cysts introduced. The number of tissue cysts in the brain of mice showed differences of 22 and 430 folds between the minimum and maximum number of cysts at weeks 7–9 and at weeks 13–14 post–infection, respectively. The variation in number of brain cysts of *T. gondii* in mice were also shown by Di Cristina *et al.*, who reported between 150 to 21000 cysts at around day 18 post infection[16].

The frequency of cysts in mice brain varies depending on the strain of mouse, the strain of *T. gondii*, the route of inoculation, and the number of organisms inoculated[7]. Moreover, there is some evidence to suggest that host genetic determinants play a major role in the production of tissue cysts in mice[17,18]. In a study by Brown *et al.* it was demonstrated that the *Ld* gene located on chromosome 17 has a role in resistance against *Toxoplasma* cysts burden, so that the load of tissue cysts in mice brain with *Ld* gene was lower than those without *Ld* gene[18]. Metabolic needs of this parasite also affect the stability of tissue cysts in the brain of hosts. For example, the CD73–generated adenosine is involved in the differentiation of *T. gondii* to lifelong cysts in the central nervous system[9]. The remarkable differences in the frequency of the brain cysts of *T. gondii*, Tehran strain are probably related to variations observed in the immunity responses of the BALB/c mice against this strain of *T. gondii*. Our results would suggest that further studies are needed to focus on the immunity response to the Tehran strain of *T. gondii* and its brain cystogenesis.

In our study, the frequency of tissue cysts in the brain of mice at weeks 13–14 was higher than in 7–9 weeks post–infection. The difference may be related to the new generations of cysts at weeks 13–14 post–infection in some mice. A difference of 3–4 folds in the size of tissue cysts observed in this study could support our opinion. However, the mechanism of regeneration of tissue cysts in mice brain has not been clearly identified. It may occur due to the leak of bradyzoites from intact cysts or the rupture of primary cysts and subsequent generation of tissue cysts[7].

There are several reports wherein the total number of tissue cysts have been counted in the brain of mice experimentally inoculated with the avirulent cyst–forming strains of *T. gondii*. In study by Berenreiterová *et al.*, the number of cysts associated with *T. gondii*, a virulent HIF strain in the brain homogenates of outbred CD1 mice was reported to be between 140 and 2 900 (mean±SD=883±938) at week 18 post–infection, a finding lower than the means obtained for tissue cysts observed in our study (mean± SEM=1 239.8±429.3) at weeks 13–14 post–infection[19]. The difference may be related to the strain of *T. gondii* or time course elapsed following the onset of infection in mice. However, the frequency of brain cysts in the study by Ferguson *et al.*[12] also mentioned in a review article by Dubey *et al.*[7], showed a different patterns following 4 weeks intervals. The number of tissue cysts in the brain of CBA/Ca mice inoculated with ME–49 strain, as reported by these researchers, was 3 720, 2 158, 3 133, and 1 538 at weeks 4, 8, 12, and 16 post–infection, respectively[7].

We used squash–smear method for microscopic counting of tissue cysts in the whole brain, which can be considered as a more precise method in comparison with counting of cysts in the samples prepared from brain homogenates[19]. In the latter method, smears are prepared from the brains squished with mortar and pestle, a technique which may lead to disruption of some cysts. Moreover, in this method the number of cysts is enumerated in a small volume of homogenate but is reported as the total number of cysts in the whole brain through calculation, a method which is prone to produce false results due to error source.

Conversely, by the squash–smear method used in our study, the cysts remained intact and the number of cysts was counted in smears prepared from the whole brains, although the method is more time–consuming.

Considering the significant differences found in the number of tissue cysts in mice brain, it seems that the Tehran strain of *T. gondii* could be considered as a suitable model for demonstrating the behavioral manipulation hypothesis of *T. gondii* in latent phase of infection. There is a body of recent evidence to suggest that the chronic infections to this parasite may be associated with brain deficiencies such as, behavioral alterations[20], deficits in learning and memory[21], personality changes[22] and as a probable cause of schizophrenia which is a chronic, severe, and disabling brain disorder with uncertain etiology[23]. In addition, as the studies on *T. gondii* show a growing trend within the recent years in Iran, our results would be helpful for Iranian researchers interested in working in the field of *Toxoplasma* and toxoplasmosis.

Based on our findings, it is concluded that the brain cystogenesis capacity of *T. gondii*, Tehran strain could demonstrate huge variations among mice with higher activity at weeks 13–14, compared to weeks 7–9, following the onset of infection. In addition, the cystogenesis capacity in cerebellum+brain stem is lower than the right

and left cerebral hemispheres.

Conflict of interest statement

We declare that we have no conflict of interest.

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