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### Original Article

## The Effect of Fluphenazine and Thioridazine on *Toxoplasma gondii* In Vivo

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#### **Abstract**

**Background:** *Toxoplasma gondii* is the most common parasite causing latent cerebral infections in human. It has been shown that some anti-psychotic drugs are able to inhibit the proliferation of the parasite in in vitro study. There is very limited data regarding the inhibitory effect of anti-psychotics on *Toxoplasma* in in vivo. In this study, we evaluated anti-*Toxoplasma* activity of fluphenazine and thioridazine drugs on *T. gondii* in mice.

**Methods:** Mice were divided into six groups: Control, sesame as vehicle, thioridazine 10 mg/kg, thioridazine 20 mg/kg, fluphenazine 0.06 mg/kg and fluphenazine 0.6 mg/kg. They were inoculated intraperitoneally with brain suspension containing tissue cysts of *T. gondii* Tehran strain. Two months after inoculation, the number of cysts in crushed smears of mice brain were counted microscopically and considered as an indicator of anti-*Toxoplasma* activity. This work has conducted in Qazvin, central Iran, 2014.

**Results:** Our study showed that fluphenazine and thioridazine could not significantly inhibit the brain cystogenesis of *T. gondii* in mice. However, the number of brain cysts was less at higher dose compared to lower doses for both drugs.

**Conclusion:** Further studies need to clear the mechanism of different structure of anti-psychotic drugs on activity of *Toxoplasma*.

## Introduction

**T***oxoplasma gondii* is an obligatory intracellular protozoan with worldwide distribution. At least, 20% of populations are seropositive for anti- *T. gondii* antibodies in most of developing countries (1), and usually 10%-20% in developed countries (2). Due to long-life persistence of brain *T. gondii* infections, the parasite is suspected as one of the possible causative agents of some of chronic neurologic disorders, particular of schizophrenia (3). There are different epidemiological and experimental evidences that support the role of *T. gondii* in developing of chronic mental disorders and behavioral alterations. These evidences have been reviewed by some of *Toxoplasma* researchers in recent years (4, 5). The most of evidences is related to schizophrenia, so that at least 40 papers reported increased seroprevalence of anti-*Toxoplasma* antibodies in schizophrenic patients with the significant differences in the most of cases (6-8).

Experimentally, there are evidences that strengthen probable role of latent *T. gondii* infections on the behavior of laboratory animals, for examples, prolongation of reaction times in mice (9), the loss of the fear response to cat odor (10), and decreasing of neophobic behavior (11). Morphologically, there are evidences that *T. gondii* infections can induce morphological changes in brain of subjects genetically predisposed. Horacek et al. reported reduction of gray matter density in *Toxoplasma* infected patients (12).

One of the most important evidences that supported the role of *T. gondii* in developing of psychotic disorders is related to effect of anti-psychotic drugs on it. The replication of *T. gondii* RH strain tachyzoites is inhibited by valproic acid (a mood stabilizer drug) in cell culture (13). This drug is able to retain innate avoidance of cat odor in rats infected with *T. gondii* (14). In addition, it had no protective effect on mice inoculated with a virulent RH

strain tachyzoites, and was inactive on brain cysts in mice inoculated with an avirulent ME49 strain of the parasite (15). Proliferation of the RH strain tachyzoites could be inhibited by anti-psychotic agents including, fluphenazine, thioridazine, and trifluoperazine (16), but there is no report in respect of inhibition of cystogenesis of the parasite in treated mice with these drugs.

In this study, we evaluated the inhibitory activity of fluphenazine and thioridazine on *T. gondii* in mice.

## Materials and Methods

### Drugs

Thioridazine was prepared from Pars Mino Industrial Co. and fluphenzazine decanoate was purchased from Chemidarou Pharmaceutical Co., Iran in injectable form. Thioridazine was dissolved in saline. Sesame oil was used for dilution and preparing suitable concentration of it. Other drugs in this investigation were xylazine (Loughrea, Co. Galway, Ireland) and ketamine (Rotexmedica, GmbH, Germany), which used for anesthesia of mice. All drugs were administrated intraperitoneally (ip).

### Animals

Sixty tree male BALB/c mice (20–25 g) were obtained from the Razi Institute (Karaj, Iran, 2014) and housed in groups of four per cage under standard laboratory conditions. They were kept at a constant room temperature ( $21 \pm 2$  °C) under a normal 12L: 12D regimen with free access to food and water. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) in such a way as to minimize the number of animals and their suffering.

### Treatment procedure

Before treatment of mice with the anti-schizophrenic agents, they were examined by

Sabin-Feldman dye test (17) for anti-*T. gondii* antibodies in order to prove to be devoid of previous infection with *T. gondii*. Mice were divided into six groups: Control (n=11), sesame as vehicle (n=10), thioridazine 10 mg/kg (n=10), thioridazine 20 mg/kg (n=10), fluphenazine 0.06 mg/kg (n=11) and fluphenazine 0.6 mg/kg (n=11). The selected doses of both drugs were based of previous animal studies that they have been used as anti-psychotic drugs. Then, these doses were not toxic for them.

Mice were inoculated intraperitoneally with brain suspension of mice which two months earlier had been infected with tissue cysts of *T. gondii* Tehran strain. A half milliliter of the brain suspension in sterile saline containing approximately 20 tissue cysts was inoculated to each of mice in all of the groups. Sesame and drugs were injected three days after inoculation of parasites, every other day for three weeks. Control mice did not receive any drugs.

At the end of second month after inoculation, the mice were anesthetized with intraperitoneal injections of ketamine/xylazine (60 mg/kg and 6 mg/kg, respectively). Mice were sacrificed under anesthesia, and their brains were quickly removed. Then, crushed smears were separately provided from whole brain of each mouse. The number of tissue cysts was counted with optical microscope with magnification 100 and 400 $\times$ . The frequency difference of the number of brain cysts between cases and controls mice was considered as inhibition index.

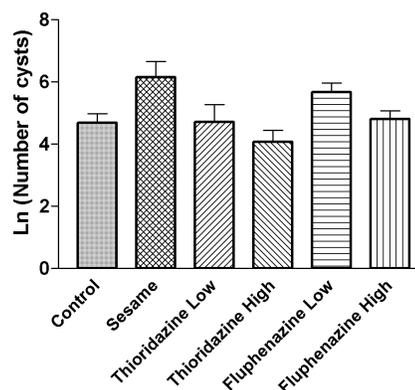
### Statistical analysis

For normalizing the initial data, natural logarithm transformation was used. In data analysis, Kolmogorov-Smirnov test, Analysis of Variance (ANOVA) and Tukey Post-Hoc were used. A level of  $P < 0.05$  was considered significant.

### Results

Before inoculation of the parasite, all of the mice were seronegative for anti-*Toxoplasma*

antibodies by Sabin-Feldman dye test. Some of the animal died in different weeks after inoculation before ending the experiments. The tissue cysts were formed in brain of all of mice, and the means number of brain cysts in the sesame group were higher than control group. However, this difference was not significant (Fig. 1). There was no different between the number of cysts at the two doses of thioridazine compared to control group ( $P > 0.05$ ) (Fig. 1). Furthermore, there was no difference between the number of cysts of two doses of fluphenazine compared to control and sesame groups (Fig. 1).



**Fig. 1:** Number of brain cysts (in Logarithm) of *T. gondii* (mean  $\pm$  SEM) in each treated and control mice groups

### Discussion

Our study showed that thioridazine and fluphenazine could not inhibit *T. gondii* in mice. These results of in vivo have a conflict with in vitro results of Goodwin and colleagues, who have reported that thioridazine and fluphenazine ( $IC_{50}$  of 1.7, 1.2  $\mu$ M, respectively) inhibited proliferation of *T. gondii* tachyzoites in in vitro study (16). Similarly, in the previous studies, the difference between the pattern of inhibitory effects of mood stabilizer valproic acid on *Toxoplasma* in in vitro and in in vivo studies has been reported (14, 15, 18). Valproic acid is active against tachyzoites of *T. gondii*

with two variable concentrations in in vitro assays. This difference might be related to their different test system (13, 18). However, valproic acid was not active against acute and chronic infections of *T. gondii* in mice (15).

On the other hand, in the clinical trial studies, treatment of schizophrenic patients with anti-toxoplasmosis drugs trimethoprim (19) and azithromycin (20) could not significantly improve the symptom of disease. This diversity of responses may be related to different efficacy of drugs in in vitro and in vivo conditions. Pharmacokinetic properties of drug including absorption, distribution, metabolism, and elimination are considered as a biological profile in in vivo models (21).

It seems that our study compared to Goodwin et al. provides a supporting evidence to confirm that anti-psychotic drugs have not inhibitory effects on tachyzoites proliferation of *T. gondii* in in vivo. Since, we injected thioridazine and fluphenazine in acute phase of infection in mice. However, they orally administered valproic acid eight weeks after inoculation *T. gondii* (in latent phase) (15). Tachyzoites of *T. gondii* proliferates inside host cells in the acute phase of infection. Then, the invasion and proliferation will be usually inhibited within less than a month and afterward, the tachyzoites convert to bradyzoites enclosed in tissue cysts that can be maintained in host tissue for long time as a chronic infection. Drugs that are used for treatment of toxoplasmosis, are effective on tachyzoites not tissue cysts.

In this study, hypothesis of inhibitory effect of fluphenazine and thioridazine on *Toxoplasma* was not established in mice. However, this finding certainly did not rebut possible role of the parasite in occurrence of chronic brain diseases especially schizophrenia. If the parasite is considered as one of the possible causative agents of schizophrenia, then, it will be prospected that *T. gondii* is caused schizophrenia only in some of infected individuals with the parasite. Since, the prevalence of *T. gondii* is much more than schizophrenia in general population. So that one third of the world's

population was infected to *T. gondii* (4), but lifetime prevalence of schizophrenia is about 1% (22).

The mechanism of anti-toxoplasmosis drugs is established. However, this mechanism about antipsychotic drugs is unclear. The typical anti-psychotic agents block D2 receptors stereoselectively for the most part of the brain, and their binding affinity is very strongly correlated with clinical anti-psychotic potency (23). In this study, we have used typical anti-psychotic drugs fluphenazine and thioridazine that block D2 receptors in the brain. An increase in intracellular calcium occurs in tachyzoites of *T. gondii* when they attach to their host cells and this increase is required for invasion. Initial attachment of tachyzoites to host cells is followed by calcium signaling (24) and this interaction was inhibited by calcium channel blockers (verapamil) and calmodulin antagonists such as trifluoperazine and calmidazolium (25). It seems that calmodulin is located in anterior portion of *T. gondii* tachyzoites, which might be related to host cell invasion (26) and it is possible by mediating calcium-dependent conoid extrusion and through calcium-stimulated secretion of lytic enzymes at the adhesion site on the host cell membrane causes to fluidize it and facilitate entry of parasite (16). Nevertheless, we did not find this effect significantly.

Another issue that should be mentioned in this study is that we have selected two doses of both fluphenazine and thioridazine less than LD<sub>50</sub>. Since, increasing dose more than it was impractical. Furthermore, in these selected doses, some mortality was happen that might be related to the difference between drugs and doses of them.

## Conclusion

Fluphenazine and thioridazine could not significantly inhibit the brain cystogenesis of *T. gondii* in mice. However, each of them reduced the percent of cysts at higher dose compared

to lower doses. However, these effects were not significant.

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