Corticosteroids Opportunist Higher *Toxoplasma gondii* Brain Cysts in Latent Infected Mice

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**ABSTRACT**

*Toxoplasma gondii* is an opportunistic zoonotic protozoan, distinguish superior brain parasite load in immune-suppressed patients. Corticosteroids are popular anti-inflammatory with immune-suppressive long course, it possible opportunist higher *T. gondii* brain parasite load and reverts encephalitis in latent infected personals. The present study concerns this concept and preferred for recognize different levels of *T. gondii* brain parasite load and immunoglobulin titers in both corticosteroids treated and untreated latent infected mice groups. A total number of 70 Swiss-Webster mice (12-18 g) were divided into four groups, the first and second ones are 30 each (infected-untreated and infected-treated group), the third and fourth 5 each (uninfected-untreated and uninfected-treated control). Administration of glucocorticoid (hydrocortisone sodium succinate) at a dose of 50 mg kg\(^{-1}\) (I.M) injection 3 times a week with oral administration of dexamethasone sodium phosphate in dose of 2.5 mg kg\(^{-1}\) day\(^{-1}\) per mouse in drinking water for sequence 2 months. The 10\(^{5}\) bradyzoites from mice brain of cystogenic ME-49 strain was used for inducing latent infected mice groups at 30 days before corticosteroids therapy. Serum and brain tissue samples were collected for serological assay and parasite load estimation from sacrificed mice. The results showed significance elevation of average percent of brain parasite load and IgM/IgG titers. All values exceeds higher and parallel to the progression of corticosteroids term in infected treated group than the infected-untreated one. In conclusion, long-term corticosteroids therapy possible opportunist higher *T. gondii* brain parasite load and induce encephalitis in latent infected murine model, imitate this serious condition in *T. gondii* infected patients who received corticosteroids therapy.

**Key words:** *Toxoplasma gondii*, corticosteroids therapy, latent infection

**INTRODUCTION**

Toxoplasmosis is an ideal latent zoonosis caused by the obligate intracellular tissue cyst-forming protozoan called *T. gondii*, which has an opportunistic character (Tenter *et al*., 2000), exceeding psychiatric and neurological medicinal impact due to fetal human cerebral toxoplasmosis, sequence to higher brain parasite load (Lovett and Sibley, 2003). The acute pathogenic tachyzoites stage induces latent brain cyst containing bradyzoites which persist viable for the rest of the host survives and possible reverted to acute tachyzoites stage due to unclear dynamic (Hassanain *et al*., 2014), the dissemination of multiplicities out-cyst zoites causes damage to adjacent brain cells, induce Toxoplasmic Encephalitis (TE) (Odaert *et al*., 1996).
Primary infection in immune-competent persons mainly occurs via one of three infective stages; consumption of either under cooked meat harboring viable bradyzoites or environmental pollution via oocysts, plus the tachyzoites materno-fetal pass. But intrinsic opportunistic relapse through bradyzoites-tachyzoites re-conversion possible set in immune-suppressed patients (Jones et al., 2001) or during pregnancy causing estradiol hormonal shift (El-Fadaly et al., 2012), abortion of pregnant female animals and women (Shaapan, 2015) and in diabetic personals (Hassanain et al., 2014), inundations series to other vague dynamics which stimulate acute encephalitis and opportunist higher brain parasite load in latent infected personals. Where, TE represents one of the most deadly cause in immune-suppressed patients through brain cells attack (Kovari et al., 2010) and deaths mostly via cystogenic types II which was the predominant chronic brain cyst forming strain (75%) in cerebral human toxoplasmosis (Zhou et al., 2004) and in edible meat of animal hosts (Elfadaly, 2007; Hassanain et al., 2011).

Anti-inflammatory corticosteroids remain the widely and the forefront drugs used to inhibit delayed hypersensitivity effects in patients with serious inflammations including both acute and chronic inflammatory signs of cerebral, congenital and ocular toxoplasmosis and increase susceptibility to infections and mask the symptoms (Hofflin et al., 1987). The interaction between T. gondii and the competent immune system does not lead to parasite elimination but to either reduction or elevation in parasite load and changes in parasite morphology and surface antigen expression (Bosch-Driessen and Rothova, 1998).

The objective of the current study is to evaluate the immune-suppressive effect of long course corticosteroids on both T. gondii brain parasite load and immunoglobulin titers in latent infected murine model, perhaps induce encephalitis series to higher T. gondii brain cysts.

MATERIALS AND METHODS
Experimental animals: A total number of 70 Swiss-Webster pathogen free mice of average weighing 18-22 g and of about 5-6 weeks old, were obtained from Laboratory Animals House, National Research Center, Egypt and used in the experiment. The animals were housed in standard environmental conditions of temperature (25-26°C) and relative humidity (50-60%) with a 12:12 light: dark cycle with standard commercial diet and water.

Study design: The total used 70 mice were divided into 4 groups; the first Infected Non-treated (IN) and second Infected-Treated (IT) groups are 30 mice of each, while the third Uninfected Non-treated (UN) and fourth Uninfected Treated control (UT) groups are 5 mice of each (Table 1).

Toxoplasma gondii strain: ME-49 T. gondii strain was maintained and secured in Zoonotic Diseases Department, National Research Center, Egypt, via 40 day inoculation-collection cycle of brain cysts in mice. Under sterile conditions, each mouse brain was homogenized in a 20 mL potter's tube and brain cysts were counted and diluted as necessary (5-20 factor dilution), ready for oral or intraperitoneal injection for continuous passage in mice (Carneiro et al., 2009).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Toxoplasma gondii infection</th>
<th>Corticosteroid treatment</th>
<th>No. of mice</th>
<th>Progress in experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN</td>
<td>+</td>
<td>-</td>
<td>30</td>
<td>3 mice sacrifice</td>
</tr>
<tr>
<td>IT</td>
<td>+</td>
<td>+</td>
<td>30</td>
<td>2, 20, 40, 50 and 60 DPCT</td>
</tr>
<tr>
<td>UN</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>Control negative</td>
</tr>
<tr>
<td>UT</td>
<td>-</td>
<td>+</td>
<td>5</td>
<td>Drug control</td>
</tr>
</tbody>
</table>

UN: Uninfected non treated, UT: Uninfected treated, IN: Infected non-treated and IT: Infected treated
Induction of latent infected mice: Mice of both IN and IT groups were infected Intra-Peritoneal (IP) by $10^3$ of mice brain cysts of *T. gondii* cystogenic ME-49 virulent strain at the day 30 prior to the onset of corticosteroids therapy and at the same time animals in UN group are still control negative, while the animals in UT group are still corticosteroids control. Chronic infected mice are confirmed by detection *T. gondii* brain cysts at 30 DPI with immunoglobulin titers (El-Fadaly *et al.*, 2012).

Samples collection: Blood samples collected and sera separated and kept at -20°C until used for serological assay. Also, brain tissue samples were collected for parasite load estimation from 3 sacrificed mice in each group at 2, 20, 40, 50 and 60 cycle to Days Post Corticosteroids Treatment (DPCT).

Corticosteroids treated mice: Two months course via synergetic action of two types of corticosteroids were used for induce corticosteroids course according the method described by Djurkoviae-Djakoviae and Milenkoviae (2001) as follows:

**Rapid onset:** Through glucocorticoid that contains hydrocortisone sodium succinate (SOLU-CORTEF®), used at a dose by 50 mg kg$^{-1}$ (I.M) injection 3 times a week.

**Long acting:** Maintained corticosteroids course by Dexamethasone sodium phosphate (DXM), oral syrup contains 0.1 mg mL$^{-1}$ (Orazone Syrup®), in a dose of 2.5 mg kg$^{-1}$ day$^{-1}$ per mouse in drinking water and was changed 3 times a week for 2 months.

Ampicillin-streptomycin (0.5 g L$^{-1}$) was used in drinking water all over the experimental course to prevent bacterial infection, with gradual reduction of corticosteroids for 7 days. Brains of dead mice during all phases of experiment were microscopically examined to confirm death due to peritonitis or encephalitis; death due to peritonitis was definite through tachyzoites isolation from peritoneal cavity, while death due to encephalitis may be referred to significant increase in the average difference of BPL between (IT and IN) groups. Survived mice were regularly sacrificed at 2, 20, 40, 50 and 60 Days Post Corticosteroid Treatment (DPCT).

Parasite load estimation: Brain tissue emulsion was prepared by homogenization with an equal volume of PBS, pH 7.4. One drop of the brain homogenate was spread on a slide and the parasite was microscopically counted in average of every 10 mL using haemo-cytometer (Dubey, 2010). The average brain parasite load (BPL/10 mL/brain) was estimated by calculating the average number of tissue cysts per brain multiplying in the average number of bradyzoites per cyst. Thus, the practical evaluation of parasite load was done by counting the total number of cyst-free bradyzoites plus in cysts bradyzoites/gm/brain in all groups. Also, the Average Differences (AD) of parasite load/10 mL/brain were recorded by subtracted the average parasite load in between the IN and IT groups (Hassanain *et al.*, 2011).

Detection of IgM/IgG titers: Antibodies of IgM and IgG were detected using ELISA diagnostic kits (VIRO, Germany), sensitivity and specificity of kits for IgM and IgG were (100 and 98.1%) and (100 and 99.1%), respectively the procedure was done according the methods described by Shaapan *et al.* (2008).
Statistical analysis: Data was statistically analyzed using the M-STAT and STATISTICA (6.0) computer programs. The average and standard deviation among the different parameters were determined as well (Godard et al., 1990). Only differences with a probability of error of less than 0.05 were considered significant.

Ethical approval: The experiments were progress according to the guide for the care and animal rights and the work is approved ethically by the Medical Research Ethics Committee-National Research Centre, Egypt under registration number 1-2/0-2-1.2012.

RESULTS
The results demonstrate reactivation of cerebral toxoplasmosis in previously *T. gondii* latent infected murine model which induced 8-week-long administration of corticosteroid drugs. Significant morbidity with typical depression and locomotor signs of cerebral toxoplasmosis in corticosteroids treated animals, followed by high mortality associated with elevated Brain Parasite Load (BPL) as compared with both healthy control and infected untreated mice. The percent of both dead mice during corticosteroids course (D1) and during corticosteroids regular reduction (D2), represented higher values (30 and 33.3%) in IT group than the corresponding (6.7 and 0%) in IN one. Also, both percent of survived mice during corticosteroids course (S1) and during corticosteroids regular reduction (S2), represented lower values (20 and 66.7%) in IT group than the corresponding (43.3 and 100%) in IN (Table 2).

ELISA IgM and IgG immunoglobulin titers were exceeding higher in infected cortisone-treated (IT) than the corresponding titers in Infected Non-treated (IN) group. The IgM curve of IT proves superior progression values in comparison with IgM of IN which were recorded sequence decline. Moreover, sero-conversion of IgM of IT group was superior value than the corresponding IgG beginning at 60 DPCT (Table 3). Also, the Average Difference (AD) of IgM was recorded higher significance values than the corresponding IgG connected with the post corticosteroids treatment days and as a long as the term of study (Table 3).

Table 2: Results of dead, sacrificed and survived mice as long as corticosteroids treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Used mice No.</th>
<th>Sacrificed mice No. (%)</th>
<th>Dead mice No. (%)</th>
<th>Survive mice (%)</th>
<th>NB</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN</td>
<td>5</td>
<td>-</td>
<td>0</td>
<td>5 (100)</td>
<td>Healthy alert animals</td>
</tr>
<tr>
<td>UT</td>
<td>5</td>
<td>-</td>
<td>0</td>
<td>5 (100)</td>
<td>Little to moderate nervous signs and depression from 5 up to 15 (DPCT)</td>
</tr>
<tr>
<td>IN</td>
<td>30</td>
<td>3X5 (15)</td>
<td>2 (6.7)</td>
<td>13 (43.3)</td>
<td>Lung congestion and heart dilatation at 37 and 42 (DPCT)*</td>
</tr>
<tr>
<td>IT</td>
<td>30</td>
<td>3X5 (15)</td>
<td>9 (30)</td>
<td>6 (20)</td>
<td>Lung congestion, heart dilatation of 5 (%) mice from 3 up to 6 (DPCT), while 6 (%) dead from 35 up to 55 (DPCT) showing sever encephalitis</td>
</tr>
</tbody>
</table>

Table 3: Number and percent of dead and survive mice during the seven days of corticosteroids withdrawal course.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Survives (%)</th>
<th>Survives (%)</th>
<th>Dead mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>UN</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>0</td>
</tr>
<tr>
<td>UT</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>0</td>
</tr>
<tr>
<td>IN</td>
<td>13 (43.3)</td>
<td>13 (100)</td>
<td>0</td>
</tr>
<tr>
<td>IT</td>
<td>6 (20)</td>
<td>4 (66.7)</td>
<td>2 (33.3) with severe encephalitis</td>
</tr>
</tbody>
</table>

UN: Uninfected non treated, UT: Uninfected treated, IN: Infected non-treated and IT: Infected treated
Fig. 1(a-f): (a) Leukocytes (white arrow) liberating ME49 tachyzoites strain (black arrow) from mice peritoneal passage after 72 h post infection -x400, (b) Typical free out cyst escaping bradyzoites from mice brain, collected from IT group at (40 DPCT)-x400, (c) Typical tachyzoites re-conversion (black arrow), with varied affected size and forms (white arrows) collected from IT group at (50 DPCT) -x400, (d) Aggregation of brain bradyzoites at the beginning of forming new cyst, collected from IT group at (60 DPCT) -x1500 and (e, f) Brain of mouse infected with 10 bradyzoites of T. gondii Me49 strain, 30 days prior to beginning of treatment with DXM which died with clinical signs of Toxoplasmic encephalitis and brain cysts collected from IT group at (60 DPCT) -x300 and 600, respectively.

Table 4: Results of both average ELISA IgM/IgG titers and the brain parasite load in both IN and IT groups, with their average difference

<table>
<thead>
<tr>
<th>Groups/Average ELISA IgM/IgG and brain parasite load (BPL/10 mg/brain)</th>
<th>IN</th>
<th>IT</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPCT</td>
<td>IgM</td>
<td>IgG</td>
<td>BPL</td>
</tr>
<tr>
<td>2</td>
<td>0.38</td>
<td>0.33</td>
<td>42.4</td>
</tr>
<tr>
<td>20</td>
<td>0.26</td>
<td>0.41</td>
<td>42.2</td>
</tr>
<tr>
<td>40</td>
<td>0.24</td>
<td>0.46</td>
<td>35.6</td>
</tr>
<tr>
<td>50</td>
<td>0.22</td>
<td>0.52</td>
<td>26.4</td>
</tr>
<tr>
<td>60</td>
<td>0.21</td>
<td>0.61</td>
<td>25.8</td>
</tr>
</tbody>
</table>


The progression elevation of average (BPL/10 mg/brain) was detected in infected cortisone-treated (IT) group and sequence decline values in Infected Non-treated (IN) one. The AD of BPL between IN and IT groups, represented higher significance curve, start to shift higher at 20 DPCT with continuous progression all over the course (Table 4). Also, the elevated AD of BPL was proved superior progression values compatible with the AD of IgM (Table 4).

The histopathological examination was existed Toxoplasmic Encephalitis (TE) in corticosteroids treated animals, where cysts number/brain always exceeding higher progression all over the course and parallel to the longer corticosteroids term (Fig. 1).
DISCUSSION

The current study set many different treatments; higher dosage and longer-term of corticosteroids. Plus, the use of *T. gondii* cystogenic type II (ME49 strain) for superior induction of brain cysts similar exist in human.

In clinical practice, corticosteroids were used for minimizing tissue damage sequence to *T. gondii* tachyzoites migration but, there is no consensuses concern defined timing and dosages during the applied Long-term therapy (Engstrom *et al*., 1991). Initiation of latent toxoplasmosis of active parasites are still present (Holand, 2014) and often presented through flare up of ocular (Benzina *et al*., 2005), cerebral (Conrath *et al*., 2003) or maternofetal toxoplasmosis (Fernandes *et al*., 2009).

As long as corticosteroids course, the results in the current study verify 30% of dead mice within IT group, while only 6.7% within IN group and vies versa in survived ones (20 and 43.3), respectively (Table 2, Fig. 1). These results signify corticosteroids therapy was the main factor maximize the number of dead mice. Also, proved depressed morbid mice with sever cerebral toxoplasmosis in IT group, manifested through developed typical locomotors signs, followed by high mortality progression to the elevated BPL which confirmed through sever histopathological TE in sacrificed and dead mice. The results coincide that experiments done in mice and rabbits studies, where the corticosteroid treated animals died sequence to higher reactive brain cysts, while untreated animals survived (Sumyuen *et al*., 1996). Also, resemble that proved by Hofflin *et al*., (1987) established a murine model of Toxoplasmic encephalitis by inducing intracerebral inoculation with *T. gondii* tachyzoites, the cortisone-treated mice died from progressive encephalitis and showed higher destruction in brain tissue than untreated ones.

A parallel possibly made with humans, where severe recurrent toxoplasmosis in elderly patients has been attributed sequence to the use of operative corticosteroids in both bone marrow transplant recipients (Peacock *et al*., 1995) and in 4 patients having recently undergone cardiac transplantation (Conrath *et al*., 2003), who are *T. gondii* sero-positive with previous history of toxoplasma retinochoroiditis. Moreover, Benzina *et al*., (2005) observe recurrent toxoplasmic retino-choroiditis after clindamycin associated with oral corticoid therapy.

Concerning ELISA sero-monitoring results of varied immunoglobulin IgM and IgG titers within the two infected groups (IN and IT), normally, IgM titer not exceeding the matching IgG titer except at the starting of either primary or opportunistic infection (Weiss and Kim, 2007; Hassanain *et al*., 2014). So, in the present study the sharp elevated IgM titer recorded superior values than the analogous IgG in IT at 45-60 DPCT. Also, the average consequences of IgM titers and their Average Difference (AD) usually in progress higher with the IT group parallel with the corticosteroids course, in contrast to the equivalent values of IgG usually in withdrawal sequences with the same group, while IgM/IgG titers pass in normal sequence with IN. This results signify powerful success of tachyzoites stage re-conversion (Fig. 1c), able to revert acute parasitemia and motivate augmented titer of IgM immunoglobulin. Also, the average difference of IgM was recorded superior significance values in IT group than the corresponding IgG and the higher shift beginning at 60 DPCT, verify success reverts of opportunistic tachyzoites to blood phase. These results were corresponding to human ocular toxoplasmosis following monotherapy with corticosteroids (Bosch-Driessen and Rothova, 1998).

The current study supports the concept of corticosteroids drugs reals with anti/protozoal drug have never given alone in *T. gondii* sero-positive patients (Damms *et al*., 1993), for controlling extra brain parasite load, diminishes nervous and psychiatric signs (Holand, 2014). Where, the fit
immune response will be successfully interacting with the outside escaping zoites (Kovari et al., 2010). Thus, only induce benign psychiatric disorders, depression or anxiety (Tenter et al., 2000). In contrast to semi-malignant course of acute cerebral toxoplasmosis possibly leading to severe neuro-psychiatric alterations particularly if associated with dopamine alteration and usually with bad prognosis and ended by death (Fernandes et al., 2009).

CONCLUSION

The present report proved that corticosteroids flare up T. gondii tachyzoites in mice harboring latent brain cysts associated with encephalitis and nervous signs, reflect the possible and parallel susceptibility in corticosteroids treated sero-positive patients, whose shouldn't be receive corticosteroids unless through a guard of systemic anti-protozoal therapy.

REFERENCES


