

Use of prednisolone to aid propagation of *Toxoplasma gondii* in mice

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Abstract. – AIM, To determine the usefulness of prednisolone in increasing the number of *Toxoplasma (T.) gondii* tachyzoites and bradyzoites in mice.

MATERIALS AND METHODS, The mice were water-fasted prior to being immunosuppressed with oral inoculation of prednisolone. Tachyzoites of *T. gondii* RH strain were inoculated into mice and the number of the parasites in the intraperitoneal fluids was then determined at 96 hs post-infection. In addition, tachyzoites of *T. gondii* ME49 strains were orally introduced into mice and the number of brain cysts formed was observed by microscopic observation at 45 days post-infection.

RESULTS, *T. gondii* propagation was found to be significantly improved by introduction of the prednisolone ($p = 0.0004$); and the number of parasite showed positive correlation with the increment in dosage of prednisolone ($r = 0.9051$).

CONCLUSIONS, The use of prednisolone greatly improved the number of parasite formed in mice: both tachyzoite and cyst forms.

Key Words:

T. gondii, Immunosuppression, Prednisolone, Tachyzoite, Cysts.

Introduction

Prednisolone is a synthetic corticosteroid that is widely used for controlling inflammation and it is known to have immunosuppressive potency. The drug is known to be used in treatment of various diseases such as acute lymphoblastic leukemia, asthma, autoimmune hepatitis, Bell's palsy, cluster headaches, Crohn's disease, dermatomyositis, Duchenne muscular dystrophy, multiple sclerosis, pyoderma gangrenosum, rheumatoid arthritis, systemic lupus erythematosus, temporal arteritis ulcerative colitis, uveitis, and vasculitis¹⁻⁷. It has been contemplated to indirectly enhance tumor growth and has been studied extensively in cancer

research^{4,8,9}. However, it is rarely been used in animals to improve yield of parasites for strain maintenance⁸.

Toxoplasma (T.) gondii is zoonotic protozoan parasite that can cause serious disease in an immunosuppressed person, but usually causes asymptomatic infection in an immunocompetent person. In this study the immunosuppressive activity of the drug was exploited for cultivation of *T. gondii in-vivo*. By deliberately immunosuppressing the host with prednisolone we rationalized that it may be possible to significantly improve the number of propagated parasites. This can facilitate studies requiring a large number of the parasites or involving *T. gondii* strains that are harder to propagate such as ME49.

Materials and Methods

Animal Ethics

Approval was obtained from University Sains Malaysia Animal Research Ethics Committee for use of mice in this study [USM/PPSF/50 (088) Jld.2].

Immunosuppression, Infection and Quantification of Tachyzoites

For the purpose of this study, only young mice that have yet to achieve sexual maturity were used. Swiss albino mice, three weeks old, were obtained and quarantined for a week in groups of three; their behavioral changes and health changes were noted during this period. Mice were weighed and prednisolone dosages were calculated based on their average weight prior to immunosuppression.

Groups of mice containing three mice each, were orally inoculated with 235 (Group 1), 470 (Group 2) and 705 (Group 3) mg/kg of prednisolone (Sigma-Aldrich, St Louis, MO, USA) by solubilizing the powdered drug in double dis-

tilled water. Three mice were included as controls; they were infected with *T. gondii* tachyzoites but were not immunosuppressed. All the mice were water-fasted overnight prior to being immunosuppressed. Infection of all mice with *T. gondii* tachyzoites was carried out 24 hours after the three groups (Groups 1, 2, 3) were treated with prednisolone. Mice were injected via intraperitoneal (IP) pathway with equal number of *T. gondii* RH tachyzoites (1×10^4 tachyzoites); the count was made using a Neubauer chamber (Merck, S.A., Madrid, Spain). Throughout the study period, observations of the mice were made on the physical signs that could indicate that they were in discomfort or distressed such as diarrhea, shuddering, lethargy and ruffled fur¹⁰. Mice were euthanized at 96 hs post infection (p.i.) after all of them were observed to be in discomfort with multiple signs as mentioned above. IP fluid was collected from each mouse in order to determine the number of tachyzoites present. The study was performed twice to ensure the reproducibility of the results.

Immunosuppression, Infection and Quantification of Bradyzoites

Two groups of three mice aged three weeks from C57BL/6 strain were used for infection with the less virulent *T. gondii* ME49 strain. One group of mice was fed 235 mg/kg of prednisolone after water-fasting overnight; while, the other group was kept as untreated control.

After 24 hours, both groups of mice were orally infected with equal numbers (1×10^3 tachyzoites) of tachyzoites of the ME49 strain. Observations were made on the mice as mentioned earlier and similar signs were looked for. The mice were euthanized 45 days p.i and their brains were harvested and placed in phosphate buffered saline (PBS). The brains were halved with a mid-sagittal cut using a scalpel; each half was placed on microscope slide, the tissue pressed firmly with a cover slip, examined under the microscope, and the number of visible cysts was counted.

Statistical Analysis

Results of RH strain tachyzoite and ME49 bradyzoite counts were expressed as mean \pm SD. The data was also analyzed with the Pearson product moment (PPM) correlation coefficient, r . In addition, t -test was also performed to compare the results of different groups of mice. $p < 0.05$ was considered statistically significant.

Results

There was a sharp increase in the average number of tachyzoites obtained in the prednisolone treated group as compared to the control group (Figure 1). The average number of tachyzoites counted per sample along with t-distribution is summarized in Table I. Figure 1 shows the

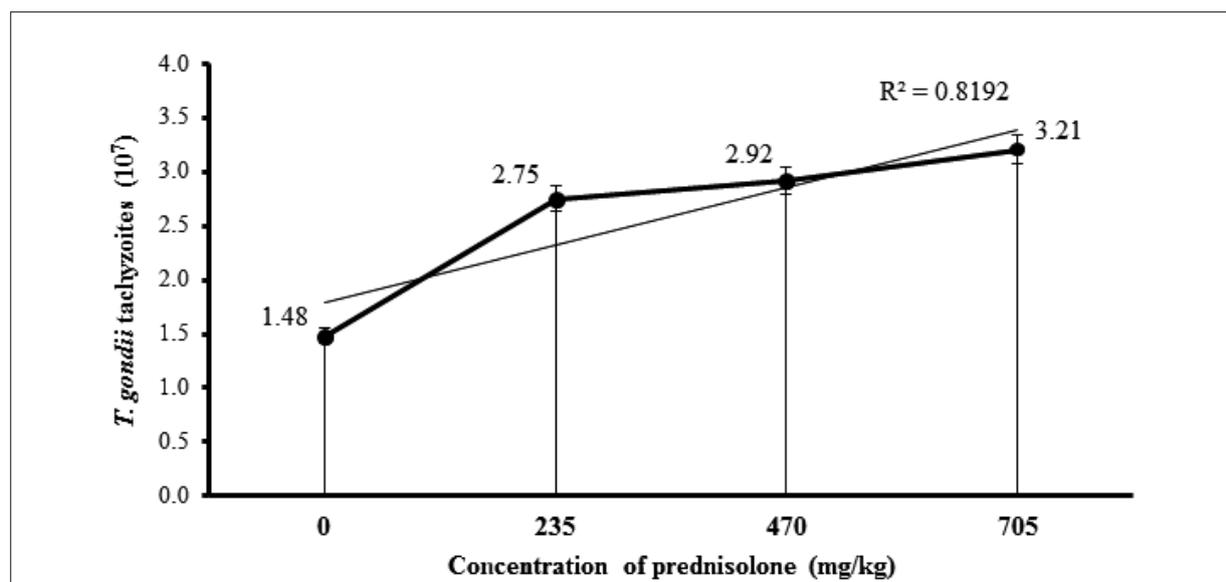


Figure 1. Number of tachyzoites vs. concentration of prednisolone fed.

Table 1. Average number of tachyzoites in each group of

Mice	Avg. tachyzoite count
Control (0 mg/kg)	$1.485 \times 10^7 \pm 0.021$
Group 1 (235 mg/kg)	$2.709 \times 10^7 \pm 0.040$
Group 2 (470 mg/kg)	$2.867 \times 10^7 \pm 0.061$
Group 3 (705 mg/kg)	$3.098 \times 10^7 \pm 0.115$

number of tachyzoites produced versus the concentration of prednisolone fed to the mouse, the PPM correlation coefficient, r , is 0.9051. Comparisons of the results between groups, using t-test, were as follows (significant value of $p < 0.05$): control and group 1 ($p = 0.0004$), group 1 and 2 ($p = 0.0080$) and between group 2 and 3 ($p = 0.0504$).

Figure 2 A shows half-brain of a mouse fed with prednisolone where ten cysts were observed as compared to two cysts in the brain of a control mouse (Figure 2 B). Analyzing all the brain-halves as mentioned above it was determined that there were 1.333 ± 0.745 cysts on the untreated group and 11.0 ± 1.414 cysts on the treated group. Comparing the results with t-test indicated that the result was, $p = 0.00002$ and the PPM correlation coefficient, $r = 1.0$.

Discussion

Prednisolone; a glucocorticoid, operates by preventing neutrophils from sticking to en-

dothelium of blood vessels; this restricts movement of neutrophils to the inflammation area¹¹. In order to achieve this, the drug down-regulates cellular adhesion molecules (CAM); these are cell surface molecules which are expressed on vascular endothelium and leukocytes⁷. Prior studies have shown significant decrease in macrophages, CD4⁺ T-cells and CD8⁺ T-cells in muscles after treatment with prednisolone⁷. CD8⁺ T-cell have been identified to be the primary mediators of resistance against acute *T. gondii* infections and also determines determine cyst number in *T. gondii* infection^{12,13}. Through removing CD4⁺ T-cells and CD8⁺ T-cells, it was observed that there was a reduction in the protective ability of lymphocytes as there was a decrease in host cell survival following infection with *T. gondii*^{14,15} and also an increase in the number of brain cyst formation in chronic infections¹⁴. CD4⁺ T-cells and CD8⁺ T-cells are also known to release gamma interferon (INF- γ), which play a significant role in controlling chronic *T. gondii* infections^{14,16,17}. Thus it can be deduced that, prednisolone causes immunosuppressive activity by decreasing both CD4⁺ T-cells and CD8⁺ T-cells; with them being vital components in immunization against *T. gondii*. It is thus conceivable that greater number of *T. gondii* was detected in the host with increase in concentration of prednisolone.

Younger mice, particularly those before the age of sexual maturity are more sensitive to the prednisolone treatment compared to matured mice¹⁸; thus, 3 weeks old mice were used throughout this

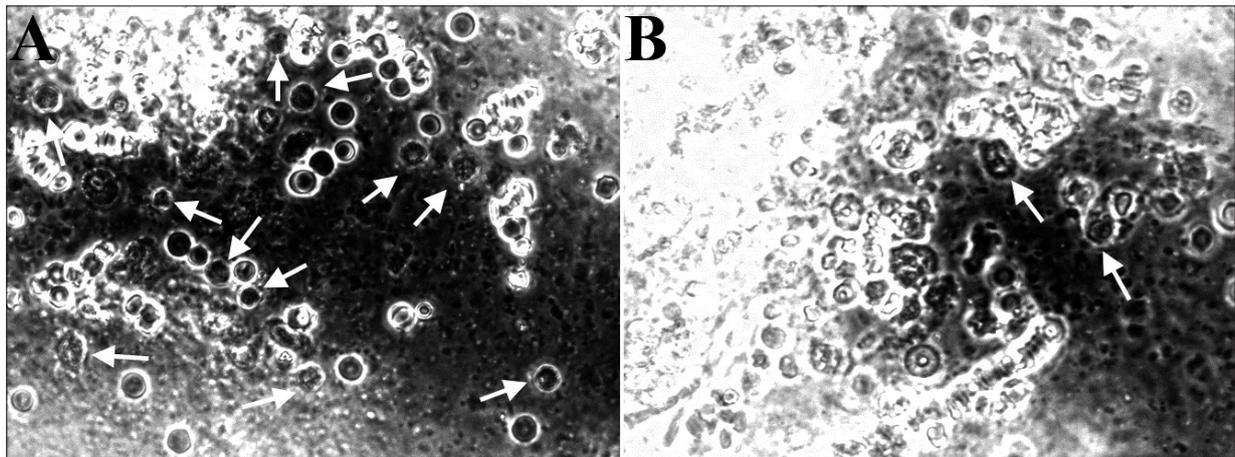


Figure 2. Microscopic image of brain sample containing *T. gondii* cysts. **A**, Immunosuppressed mice **(B)** control mice. Brain image was obtained by dividing an infected brain into two halves, fixing them under two slides and visualizing with Zeiss Axio Observer Z1 microscope (400 \times). (Arrows indicate *T. gondii* cysts).

study. The dose of the prednisolone used in this study was based on the previous toxicity report which showed that LD 50 in mice is 1680 mg/kg, and that an oral dose of up to 705 mg/kg was safe to be administered to the mice 24 hours prior to *T. gondii* infection.

The choice of mice and the method of infection are important considerations in the study on bradyzoites. Swiss albino mouse strain is susceptible to chronic infection through type I *T. gondii* but are resistant to type II infection; thus, they are not suitable to study cyst formation in murine brain¹⁹. Thus C57Bl/6 strain of mouse was used in the second part of this study as it is susceptible to chronic infection by type II strain. They are also only susceptible when infected orally¹⁹.

Statistically, the high correlation coefficient of the PPM indicates a strong positive linear relationship between the drug concentration and the number of tachyzoites produced. This supports our hypothesis that the drug helps to increase the propagation of the parasites in mice. This hypothesis is further supported by the *t*-test results where the difference between the control group and group 1 showed significant difference ($p < 0.05$). Similarly, there was also significant difference between group 1 and group 2; but, the difference was not significant ($p > 0.05$) between group 2 and group 3. Therefore, usage of 235 mg/kg dose of prednisolone is sufficient for cultivation of *T. gondii* in mice host as there was a strong significant difference in number of parasites when compared to untreated control group. Furthermore, immunosuppressing the mice with very higher dose of prednisolone make them more susceptible to other infections. Therefore, immunosuppressing the mice with 470 mg/kg, although produced significantly greater number of parasites than 235 mg/kg, is not recommended.

It is also noted that mice fed with 235 mg/kg of the drug yielded significantly ($p = 0.0002$) more cysts in C57BL/6 mice compared to the mice that were not immunosuppressed. PPM correlation coefficient was expected, where a strong positive linear relationship between the drug concentration and the number of cysts produced was observed. Thus, prednisolone improved the number of both tachyzoites and bradyzoites in infected mice. Therefore, prednisolone can be used to propagate a greater number of the parasite from both virulent (RH, type I) and avirulent (ME49, type II) strains in mice.

Conclusions

The use of 235 mg/kg prednisolone to immunosuppress mice prior to infection with *T. gondii* can help propagate a greater number of tachyzoites or bradyzoites for use in further studies or for strain maintenance.

Acknowledgements

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