PREVENTION OF SHEDDING AND RE-SHEDDING OF TOXOPLASMA GONDII OOCYSTS IN EXPERIMENTALLY INFECTED CATS TREATED WITH ORAL CLINDAMYCIN: A PRELIMINARY STUDY

A. Malmasi1, B. Mosallanejad2, M. Mohebali3, M. Sharifian Fard1 and M. Taheri4

1 Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2 Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Ahvaz, Ahvaz, Iran
3 School of Public Health, Medical Sciences/University of Tehran, Tehran, Iran
4 Rastegar Research Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Impacts

• Giving Clindamycin to cats prevented them from shedding oocysts of Toxoplasma gondii when experimentally infected.
• Immunosuppression of cats previously shedding oocysts made them re-shed.
• The cats that received Clindamycin did not shed oocysts even under severe immunosuppression.

Keywords: Clindamycin; toxoplasmosis; Toxoplasma gondii; oocyst shedding; cat; dexamethasone

Correspondence:
A. Malmasi. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, P.O. Box 14155-6453, Tehran, Iran. Tel.: +98 21 66920035; Fax: +98 21 66933222; E-mail: malmasia@vetmed.ut.ac.ir

Received for publication February 12, 2008

Summary

This work aimed to evaluate the effects of preventive oral Clindamycin in cats infected with Toxoplasma gondii. Twelve short hair cats were divided into two groups (group 1 and group 2). No titres of T. gondii antibodies were detected in these cats before the experiment. The animals from group 1 were infected with tissue cysts of T. gondii and group 2 were infected and treated with Clindamycin (20 mg/kg/day). The infection was done with almost 40–50 tissue cysts for each cat on day 0. The cats from group 2 were treated with Clindamycin by oral route for 24 days (from day 3 to day 21). At day 45, the groups 1 and 2 were divided into two subgroups with three animals each. Subgroups 1A and 2A were immunosuppressed with dexamethasone (1 mg/kg/day) for 30 days and subgroups 1B and 2B were not immunosuppressed. Faecal exam looking for oocyst shedding was made by 30 days after T. gondii infection, and for 30 days after immunosuppression. All kittens from group 1 shedding oocysts after infection, while animals from group 2 did not shed. After immunosuppression period, all animals from group 1A re-shed oocysts and animals from group 2A remained without shed. However, 2 (66.6%) of the kittens from subgroup 2B shed oocysts 19–20 days after re-challenge. Based on this preliminary study, Clindamycin had a complete inhibitory effect on shedding of oocysts by cats, even under severe immunosuppression, which is a new finding not reported elsewhere.

Introduction

Toxoplasma gondii is a protozoal parasite with a worldwide distribution that infects most mammalian species, including cats and humans. Cats infected with T. gondii may shed millions of oocysts (Dubey, 2002). As humans, especially children, pregnant seronegative women and immunocompromised individuals may become infected with T. gondii oocysts (Greene, 1998, 2006), there is a need for medications capable of preventing the shedding of oocysts by cats. Many medications have been tried to inhibit oocyst shedding (Frenkel, 1974; Dubey and Yeary, 1977; Frenkel and Smith, 1982; Rommel et al., 1988). However, the frequent side effects and variable clinical
efficacy of these medications have led to the investigation of alternative antiprotozoal therapy (Davidson et al., 1996).

In this trial, we studied preventive oral Clindamycin therapy in cats. We also immunosuppressed the cats to evaluate the effect on their shedding and re-shedding. Furthermore, we re-infected some of these kittens to evaluate the role of Clindamycin in the shedding of kittens when re-exposed.

Materials and Methods

Experimental infection of mice
Syrian mice (Mus musculus) were inoculated intraperitoneally with a suspension containing tissue cysts of the Tehran strain of T. gondii, which cause subacute infections in both mice and kittens. The mice were killed after 30 days and their Cerebral infections were confirmed by the observation of T. gondii tissue cysts in tissue smears under light microscopy (×40). The infected brains were homogenized with normal saline to make an oral suspension to be fed to the kittens.

Experimental infection of kittens
Twelve healthy kittens of both gender aged 1.5–2 months with average body weights of 650–900 g, were selected, examined, vaccinated against feline viral diseases (Herpes, Calici and Panleukopenia viruses) (Fel-o-Vax, FortDodge, IA, USA) and dewormed with Mebendazole tablets (Rouz Darou Co.,Tehran, Iran). The cats were healthy and had no detectable specific anti-T. gondii titres by IFAT and no parasites, particularly coccidian oocysts in the stool exams. All the Kittens received one equal amount (2 ml) of homogenized suspension of mice brain tissues containing almost 40–50 T. gondii tissue cysts. The kittens were divided into two groups of six (groups 1 and 2). Kittens from group 1 were infected with T. gondii tissue cysts (Positive control) and kittens from group 2 were infected and treated with Clindamycin (Dalacin; Pharmacia, Puurs, Belgium) (20 mg/kg/day) from day –3 to 21 of the infection; the medication was filled in small capsules and then fed to the kittens.

At day 45, the groups 1 and 2 were divided into two subgroups with three animals each. subgroups 1A and 2A were immunosuppressed with dexamethasone (1 mg/kg/day) parenterally for 30 days and subgroups 1B and 2B were not immunosuppressed. At the end of trial, the kittens from subgroup 2B were re-infected by the same method and were monitored for oocyst shedding on a daily basis for another 30 days.

Body weights of the kittens were measured before and at the end of the experiment.

Haematological analysis
Blood samples were collected from each kitten. Antibody titres, serum chemistry and certain haematological values such as PCV (conventional microhaematocrit), Leucocytes and Erythrocytes (Neubauer haemocytometer; Karl Hecht KG, Sondheim, Germany) were measured and counted before and after the experiment. The biochemical factors were measured using standard methods (BUN: GLDH, SCr.: Jaffe, ALT and AST: IFCC and ALP: DGKC).

IFA test
Serum samples were taken before and after the trial for IFAT (Lappin, 1996).

Faecal examination
Faecal exam looking for oocyst shedding was made by 30 days after T. gondii infection, and for 30 days after immunosuppression. Finally, the kittens from subgroup 2B had faecal exams for an additional 30-day period after the re-challenge. The faeces using concentration technique (Burney et al., 1999) were examined for the oocysts under low magnification (×40).

Statistical methods
The statistical analysis was performed with spss (version 13.0; SPSS Inc., Chicago, IL, USA). For the comparison of biochemical, haematological and body weights values of two groups, independent t-test was used. P-value < 0.05 was considered significant. Geometric Mean Reciprocal Test (GMRT) for comparing anti-T. gondii antibody titres in both groups was used.

Results
All kittens from group 1 (positive control), shed oocysts after 7–8 days, which lasted 7–8 days, while none of the kittens from group 2 shed any oocysts up to 30 days post-infection. There were no significant differences in the values of BUN, serum creatinine, ALT, ALP, AST, mean haematological values and mean body weights between two groups in independent t-test (P = 0.07). No titres of T. gondii antibodies were detected in these kittens before the experiment. The antibody titres of all the cats at the end of trial ranged from 10 to 1280 (titres of 20 and lower that considered as negative). On day 30 post-infection, IFAT results for group 1 showed a relatively high rise in contrast to that of group 2 (GMRT: 564 versus 22).

After immunosuppression period, all animals from subgroup 1A re-shed oocysts after 20–21 days for 9–10 days,
while animals from subgroup 2A remained without shed. None of the kittens from either subgroups 1B or 2B, shed any oocysts. However, after re-challenging the latter subgroup, two of the kittens (66.6%), re-shed oocysts after 19–20 days.

**Discussion**

Chemoprophylactic medications may be beneficial in treating cats owned by pregnant women to reduce the risk of potential exposure of the foetuses to oocysts (Greene, 1998, 2006). Our limited descriptive study showed that giving Clindamycin prophylactically to cats made them oocyst-free and as long as they were receiving the medication, they did not shed oocysts even under severe immunosuppression. This is a new finding that has not been reported elsewhere; 100 mg/kg of parenteral Clindamycin led two cats to shed 2.5 times fewer oocysts than the controls (Dubey and Yeary, 1977). Oral Clindamycin in two groups of cats with nearly half of our dose suppressed the shedding of *T. gondii* oocysts (Davidson et al., 1996); however, the cats were not immunosuppressed. Immunosuppression with high doses (10–80 mg/kg) once a week of Prednisolone made chronically infected cats to re-excrete oocysts (Greene, 2006). In our study, 1 mg/kg/day of dexamethasone made non-treated cats to re-excrete; however, it did not have the same effect on Clindamycin treated cats. This shows high efficacy of Clindamycin. Clindamycin was well tolerated by our kittens with no common reported side effects (Greene, 2006). Results of IFAT were consistent with the shedding of oocysts, i.e. kittens that had shed oocysts (group1) had higher titres of antibody as well. Re-shedding of the subgroup 2B (which were not immunosuppressed and did not shed initially) after re-challenge seems logical, as because of the highly coc-cidiocidal effects of Clindamycin, they were almost seronegative, thus highly susceptible to intestinal replication and shedding. It would have been better if we had re-challenged subgroup 1B to evaluate the relationship between the previous exposure to the parasites (seropositivity) and re-shedding after re-exposure, but unfortunately we did not.

Our findings are just a model and further experiments with larger groups are needed.

**Acknowledgements**

We would like to appreciate A. Aliari and M. Kanani for their outstanding technical assistance. The experiments in this study comply with the animal research regulations of the country.

**References**


