

The Effect of Betamethasone and IFN- γ on Replication of *Toxoplasma gondii* (RH Strain) and Nitric Oxide Production in HeLa Cell Culture

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Received: 26 October 2005; Received in revised form: 1 February 2006; Accepted: 27 March 2006

ABSTRACT

Toxoplasmosis is a protozoal infection caused by *Toxoplasma gondii*. Toxoplasmosis produce severe damage in patients who are immunosuppressed. In those who are immunosuppressed, latent infection can be reactivated resulting in acute disseminating disease. Betamethasone is a synthetic glucocorticoid, used as an anti-inflammatory and immunosuppressant in a wide variety of disorders. The aim of this study was evaluation of betamethasone as an immunosuppressor drug on infected cells by *Toxoplasma gondii*. In this study, at first HeLa cells were grown in 24 well culture plates in culture medium. When confluent monolayer was obtained, we compared 6 groups to evaluate the effect of betamethasone as a corticosteroid drug (two concentrations 4 and 40 $\mu\text{g/ml}$) and the effect of IFN- γ (100 IU/ml) on growth, replication and Nitric Oxide (NO) production. The results showed, that high number of plaques were seen in group with 40 $\mu\text{g/ml}$ of betamethasone and the lowest number of plaques were seen in group with 100 IU of IFN- γ . The difference between plaque number in control and groups treated with IFN- γ and betamethasone was significant ($P < 0.05$). The groups with betamethasone or IFN- γ without tachyzoites did not show any effect on cell structures. Replication rates in the wells treated with IFN- γ were decreased significantly 72h post inoculation in comparison with control group ($P < 0.05$). There was no significant difference among different groups in NO production. The results indicated that betamethasone increase the invasion of tachyzoites to host cells in vitro.

Key words: Betamethasone; HeLa cell; IFN-Gamma; Replication; *Toxoplasma gondii*

INTRODUCTION

Toxoplasmosis is a zoonotic infection caused by *Toxoplasma gondii*. Approximately 500 million people

have antibodies against the causative organism *T. gondii*.¹ In the immunocompetent individual the disease is usually asymptomatic and self limiting.² However, in those who are immunosuppressed as a consequence of chemotherapy, latent infection can become reactivated resulting in acute disseminating disease with neurological symptoms.³

Betamethasone is a synthetic glucocorticoid, used as an anti-inflammatory and immunosuppressant in a

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wide variety of disorders. Betamethasone has stimulatory effect on surfactant protein B gene transcription as expressed by surfactant protein B messenger RNA accumulation.⁴ On the other hand IFN- γ can inhibit the parasite growth in vitro condition. The result of a study conducted by Nagineni et al indicated that, treatment of human retinal pigment epithelial cells (RPE) with IFN- γ in vitro condition can inhibit the parasite growth, by starving *T.gondii* from L-tryptophan, an amino acid which is essential for growth.⁵ IFN- γ induces the upregulation of indoleamine-2,3 dioxygenase (IDO) which converts L-tryptophan to kyhurenine.

In another study conducted by Brunton et al it was shown that pre – treatment with IFN- γ inhibited, but did not completely halt the growth of *T.gondii* tachyzoites by rRVE cells up to 24 h past inoculation.³ Parasite replication was significantly inhibited at 48, 72 and 96 h post inoculation as compared to control.

They also showed that rRVE cells treated with IFN- γ appear to be capable of restricting *T.gondii* replication by a Nitric Oxide (NO) independent mechanism.³ The present study was conducted to evaluate effect of betamethazone and IFN- γ alone or in combination on growth rate and replication of *T.gondii* in Hela cell culture .NO production in the cell culture was also evaluated .

MATERIALS AND METHODS

Parasite

Tachyzoites of *T.gondii* (RH strain) was injected intraperitoneally in mice . The mice were killed after 3 days and the tachyzoites were collected from peritoneum.³

Cell Culture

Hela cells were grown in 24 well flasks in DMEM medium with 10% FCS(Gibco) at 37°C in 5% CO₂. Following monolayer formation, the medium discarded and free serum medium was replaced.⁶

Tachyzoites of *T.gondii* were cultured in vitro in HaLa cells . Betamethazone (4 and 40 μ g/ ml) as well as IFN- γ (100 IU /ml) (Bohringer®, Germany)were added to the wells .Hela cell culture(without any betamethasone, IFN- γ and tachyzoites), and Hela cell culture with betamethasone or IFN- γ were also evaluated.

In days 1,2 and 3 after adding the tachyzoites, supernatants were collected and the plates checked for plaques/field by inverted microscopy, *T.gondii* replication was assessed by counting the number of tachyzoites obtained from harvested Hela cells , 3 days post inoculation.

NO Measurement

For NO measurement, supernatant was collected daily and compared with eight nitrite standard concentrations (0 to 35 μ M). 100 μ l of each of the standards and samples (supernatant of cell culture) was added into the wells of a 96-well plate in duplicates, then 100 μ l of Griess reagent was added to each well and the plate incubated at room temperature for 10 min. The absorbance was read at 550 nm on a platerereader.⁹

Statistics

For analysis of differences of parasite replication, plaque/field and NO production among different groups, anova-oneway test was used.

RESULTS

Effect of Betamethasone and IFN - γ on Plaques/Field Production

The plaques were counted on each field after 72h post inoculation by inverted microscope (400x). Mean and SD were calculated for 7 experiments (Table 1).

Table 1. Effect of IFN- γ and betamethasone on production of plaque/ field (\times 40) and replication of *Toxoplasma gondii* in Hela cell culture.

Groups	No. of plaque / field (\times 40) (Mean \pm SD)	No. of parasite $\times 10^4$ / ml (Mean \pm SD)
IFN- γ (100 IU/ml)	1.14 \pm 0.62	45 \pm 1.8
Betamethasone (4 μ g/ml)	4.00 \pm 0.81	94 \pm 3.8
Betamethasone (40 μ g/ml)	5.12 \pm 0.83	162 \pm 4.5
IFN- γ (100 IU/ml) + Betamethasone (4 μ g/ml)	1.85 \pm 0.89	63 \pm 3.74
IFN- γ (100 IU/ml) + Betamethasone (40 μ g/ml)	3.08 \pm 1.15	111 \pm 8.5
Control	2.24 \pm 0.69	79.2 \pm 3.5

Effect of Betamethasone and IFN- γ on *T. Gondii* Replication



Figure 1. Monolayer of HeLa cell culture 72 h after addition of Betamethasone (40 µg/ml).

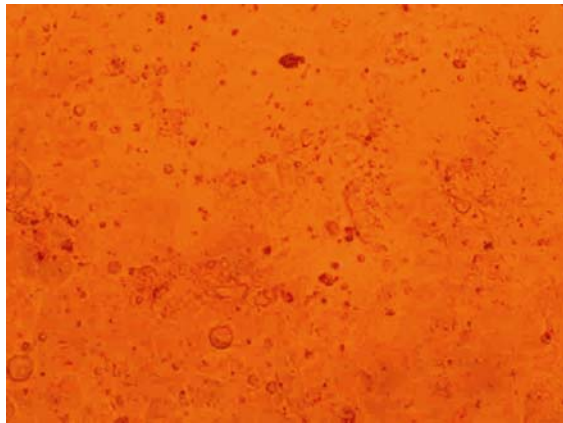


Figure 2. HeLa cell culture, tachyzoites of *Toxoplasma gondii* 72 h after addition of Betamethasone (40 µg/ml)

The highest number of plaques were seen in group with 40 µg/ml of betamethasone (5.12±0.83 plaques/field) (Figure 2) and the least number of plaque were seen in group with 100 IU of IFN- γ (1.14±0.62 plaques/field). The difference between plaque number in control group and groups treated with IFN- γ and betamethasone was significant ($P < 0.05$). On the other hand, betamethasone or IFN- γ without tachyzoites did not show any effect on cell structure of the cultured cells (Figure 1).

Effect of Betamethasone and IFN- γ on Parasite Growth and Replication

T.gondii tachyzoites in HeLa cells culture were counted after 72h post inoculation. The parasite growth and replication rates in the wells treated with IFN- γ were decreased significantly 72h post inoculation in comparison with control group ($P < 0.05$) (Table 2).

Table 2. Effect of IFN- γ and betamethasone on production of NO by HeLa cell infected with *Toxoplasma gondii* in cell culture.

Groups	NO production (µmol / ml) (Mean± SE)		
	After 24 hours	After 48 hours	After 72 hours
IFN- γ (100 IU/ml)	2.83± 0.48	3.02± 0.91	2.92± 0.68
Betamethasone (4µg/ml)	2.29 ± 0.71	2.35± 0.78	2.63± 0.42
Betamethasone (40µg/ml)	2.05± 0.48	2.16± 0.90	1.83± 0.62
IFN- γ (100 IU/ml) + Betamethasone (4µg/ml)	2.50± 0.63	2.27± 0.44	2.33± 0.27
IFN- γ (100 IU/ml) + Betamethasone (40µg/ml)	2.14± 0.76	2.17 ± 0.11	2.09±0.57
Control	2.43± 0.16	2.98 ±0.43	3.10 ±0.81

Effect of Betamethasone and IFN- γ on NO Secretion

The amount of NO production after 24, 48 and 72h post inoculation in HeLa cell treated with IFN- γ and betamethasone are shown in Table 2. There was no significant difference among different groups in NO production.

DISCUSSION

Previous studies have shown that *T. gondii* can replicate in HeLa cell culture.^{7,8} According to the present study, betamethasone and IFN- γ can affect the growth and replication of *T. gondii* in HeLa cells by a NO independent mechanism.

Betamethasone can increase and IFN- γ could decrease the replication of *T.gondii*. Brunton et al showed that, the cytokines can affect the replication of *T.gondii* in rat retinal vascular endothelial (rRVE) cells.³ But the effect of cytokines on the replication of *T. gondii* in rRVE cells and the mechanisms that may be involved have not been investigated. Schluter et al have shown that NO could have a smaller role in long term of latent *T.gondii* infections than previously considered. These findings indicated that a long term chronic infection in resistant animals was maintained by a NO independent mechanism. IFN- γ treated rRVE

cells appear to be capable of restricting *T. gondii* replication by a NO independent mechanism.¹⁰

Naginei et al found that pretreatment of cultures with recombinant human tumor necrosis factor alpha, alpha interferon IFN- α , IFN- β or IFN- γ for 24 h prior to inoculation inhibited *T. gondii* replication in a dose dependent manner. Of these cytokines, IFN- γ was the most potent, and *T. gondii* replication was completely inhibited at a concentration of 100 U/ml. The anti-toxoplasmodic activity of IFN- γ was significantly blocked by monoclonal antibody to IFN- γ . Treatment of the cultures with IFN- γ from day 1 or 2 postinoculation with *T. gondii* also offered protection against the parasite.

The anti-toxoplasmodic activity of tumor necrosis factor alpha or IFN- α , IFN- β or IFN- γ in these cultures was found to be independent of the nitric oxide (NO) pathway.¹¹

Halonen et al found that gamma interferon (IFN- γ) was the main cytokine preventing reactivation of *Toxoplasma* encephalitis in the brain. Microglia are important IFN- γ -activated effector cells controlling the growth of *T. gondii* in the brain. IFN- γ can also activate astrocytes to inhibit the growth of *T. gondii*. In wild type astrocytes *T. gondii* growth was significantly inhibited by IFN- γ .¹²

In addition, betamethasone can increase the invasion of tachyzoites, so more cell destruction could be seen following addition of betamethasone to the cell culture infected with tachyzoites. Whereas betamethasone alone did not any effect on the structure of the cell culture that were not infected with any tachyzoites.

In conclusion, it could be suggested that, individuals under long term treatment of betamethasone need more care and should be protected against *T. gondii* infection and if these individuals are infected with *T. gondii*, IFN- γ maybe used to reduce the invasion of tachyzoites.

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