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Original Article

The immunomodulatory effects of rolipram abolish drug-resistant latent phase of *Toxoplasma gondii* infection in a murine model

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ABSTRACT

Background: Latent toxoplasmosis always has the risk of reactivation leading to significant sequelae. The available medications, for chronic toxoplasmosis, are awfully limited by resistance of *Toxoplasma* cysts. Therefore, there is a growing necessity for novel therapeutic approaches. Agents increasing cAMP levels and downregulating proinflammatory cytokine could inhibit *Toxoplasma* conversion to the bradyzoite stage. This study explores a potential immunomodulatory effect of rolipram, a PDE4 inhibitor, on the course of experimental toxoplasmosis and links this role to deterrence of the resistant chronic phase of the disease.
Materials and methods: Mice infected with low pathogenic strain of *Toxoplasma gondii* were treated with rolipram for three weeks. The effect of rolipram was evaluated through tissue injury scoring, brain cyst count, specific IgG titers as well as TNF-α, IFN-γ and IL-12 assays.
Results: Rolipram was partially able to prevent the progression to chronic toxoplasmosis. *Toxoplasma* brain cyst burden showed a 74% reduction while *Toxoplasma*-induced inflammatory foci per liver area and nucleated cells per inflammatory focus were significantly reduced: 57.14% and 61.3% respectively. Significant reduction of TNF-α (84.6%), IFN-γ (76.7%) and IL-12 (71%) levels was demonstrated along with significant inhibition of anti-*Toxoplasma* antibody response.
Conclusion: Rolipram efficiently modulated the *Toxoplasma*-induced immunological changes with a consequent remission of chronic toxoplasmosis. This study is the first to report the utilization of PDE4 inhibitors as possible immune modulators of chronic phase of *Toxoplasma* infection.

1. Introduction

Toxoplasmosis is a cosmopolitan infection caused by *Toxoplasma gondiii*, an obligatory intracellular parasite. It is estimated that 30–50% of the world population are infected with the parasite, representing one of the most prevalent infections among humans [1]. Latent toxoplasmosis always has the risk of reactivation, in immunocompromised patients, leading to acute encephalitis [2] or relapsing ophthalmitis in immunocompetents [3]. Current therapy relies on agents that can suppress active infection with no effect on the latent phase of the disease [4]. The available medications are awfully limited by resistance of *Toxoplasma* cysts and drug toxicity to the children, pregnant

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women and immunocompromised patients [5]. Interference with the pathways mediating tachyzoite–bradyzoite interconversion is crucial for modulating the pathogenesis of *Toxoplasma* infection.

Cyclic nucleotide phosphodiesterases (PDEs) are critical modulators of cellular levels of cAMP catalyzing cyclic nucleotide hydrolysis [6]. Agents causing high cAMP levels have been reported to inhibit *Toxoplasma* conversion to the bradyzoite stage [7]. PDE4 inhibitors could interfere with tachyzoite–bradyzoite interconversion due to suppression of cytokines; TNF-α, IFN-γ and IL-12, having pivotal roles in this transition [8,9]. While proinflammatory cytokines, especially IFN-γ and TNF-α, are critical for an effective checking of *Toxoplasma* growth and dissemination, they are damaging when overproduced [10]. Complete neutralization or inhibition of pro-inflammatory cytokines could lead to an exacerbated acute disease. Neutralization of IL-12 has shown to result in an overwhelming *Toxoplasma* proliferation and a severe acute stage in infected mice [11].

This study represents an alternative approach for better management of latent toxoplasmosis. The purpose of the study was to explore a potential role of rolipram, a PDE4 inhibitor, in aborting the progression to chronic toxoplasmosis. The roles played by pro-inflammatory cytokines to mediate this progression were highlighted.

2. Materials and methods

2.1. Animals

Female Swiss albino mice, 6–8 weeks old and weighting 25–30 g (from a local KAU university vendor) were kept under standard laboratory conditions. The mice had free access to standard diet and water throughout the experiment. Animal experimentations were performed in accordance with the Code of Ethics of EU Directive 2010/63/EU for animal experiments [12]. Mice were divided into three groups (10 mice each). An infection control group (GI) were challenged with *T. gondii* (KSU strain). The second group (GII) received, day 7 post infection and for three weeks, 10 mg/kg/day rolipram (Sigma, St. Louis, MO, USA) by oral gavage. A drug-control group (GIII) received rolipram (as in GII) without a parasitic challenge. A single rolipram concentration representing the highest but safe therapeutic dosage [13], was used in the study.

2.2. Parasitic challenge

Mice of GI and GII were challenged with intraperitoneal (i.p.) injection of 20 *Toxoplasma* tissue cysts in a total volume of 0.1 ml sterile brain emulsion of a Swiss albino mouse infected two months earlier. The low pathogenic, cyst-forming KSU strain of *T. gondii* (a generous gift from Ain-shams Diagnostic and Research Unit, Cairo, Egypt) was used.

2.3. Rolipram preparation

Rolipram was initially dissolved, at a concentration of 1 mg/10 μl DMSO. A final concentration of 1 mg/ml was reached by dilution with an appropriate volume of phosphate-buffered saline (PBS). The final concentration of the vehicle (DMSO) was 1%. Mice of relevant groups were treated with rolipram as mentioned above.

2.4. Blood and tissue sampling

On day 14 post-infection (PI), 3 mice/group were anesthetized and then euthanized by cervical dislocation. Liver excision and blood sampling were done using standard procedures. Blood sampling was repeated at day-28 PI (after completion of rolipram treatment), for the rest of mice (7 mice/group). On day 50 PI, 7 mice/group were euthanized and similar processes of blood sampling and brain harvesting were performed.

Blood samples were centrifuged, sera were separated and then stored at −80 °C for later analytical assays. Recovered brains were immediately homogenized for cyst counting. Liver samples were fixed in buffered formalin (10%) for histological examination.

2.5. Brain cyst counting

Isolated brains, from all mice groups (day-50 PI), were crushed individually in a mortar. The grinded brain tissue was dispersed in 2 ml saline by passing it through needles with decreasing gauge sizes. The number of cysts, per individual brain, was then counted microscopically in 10 ml of the brain emulsion and the entire number of cysts per brain was calculated.

2.6. Histopathological examination

Formol-fixed liver samples, harvested on day-14 PI, were immersed in paraffin, cut into 5 μm-thickness sections and stained with hematoxylin and eosin. The liver sections were assessed microscopically for inflammatory foci and nucleated cells. Inflammatory foci, defined as isolated cluster of 6–60 nucleated cells, were quantified as previously described [14]. They were counted, at a magnification of ×100, in 8 mm^2 surface area of liver tissue. Nucleated cells/inflammatory focus were counted at a ×400 magnification.

2.7. Antibody assays

Dye test (DT) was performed on serum, recovered from all remaining mice (7/group) at day-50 PI. A micro-modified method of Sabin-Feldman assay was performed [15]. In a microtiter plate, a mixture of 2×10^6 tachyzoites in 50% accessory factor was added to assigned volumes of mice sera to get serial four-fold dilutions. Tachyzoites, of RH strain, were freshly recovered by peritoneal lavage of mice infected two days earlier while accessory factor was a *Toxoplasma*-negative serum checked for specific antibodies in a previous dye test. After 1 h incubation at 37 °C, titers were read as the sera dilutions showing 50% parasite killing. End-points were determined, after the addition of methylene blue, using a phase contrast microscopy. Titers ≥1/16 were considered positive.