Available online at www.sciencedirect.com
SCIENCE DIRECT®
Microbes and Infection 7 (2005) 49–54
Microbes and Infection
www.elsevier.com/locate/micinf

Original article

Stage conversion of *Toxoplasma gondii* RH parasites in mice by treatment with atovaquone and pyrrolidine dithiocarbamate

Olgica Djurković-Djaković *, Aleksandra Nikolić, Branko Bobić, Ivana Klun, Anastasija Aleksić

Toxoplasmosis Research Laboratory, Department of Medical Parasitology, Institute for Medical Research, Dr. Subotića 4, P.O. Box 102, 11129 Belgrade, Serbia and Montenegro

Received 7 July 2004; accepted 24 September 2004

Available online 15 December 2004

Abstract

The mouse-virulent RH strain of *Toxoplasma gondii* is generally considered to have lost its cyst-forming capacity, and conversion of RH tachyzoites into cysts in non-immune mice has previously been shown exclusively following early treatment with sulfadiazine (SDZ). We here describe the development of tissue cysts in mice infected with RH strain parasites and treated with atovaquone (ATO) combined with pyrrolidine dithiocarbamate (PDTC). Groups of Swiss-Webster mice infected intraperitoneally (i.p.) with 10^2 RH tachyzoites were treated with 5, 25 and 100 mg of ATO/kg per day alone or combined with PDTC at 250 mg/kg per day from day 1 postinfection (p.i.) for 14 days. A total of 19 mice survived the 6-week observation period. Of these, brain cysts were recovered in nine (47%), with burdens ranging from 50 to 3120 (mean ± S.D. = 622 ± 963). All cyst-harboring mice had high specific IgG antibody levels (1:10,240–1:40,960, corresponding to 500–2000 IU/ml), as did one mouse in which cysts were not demonstrated, which was therefore included in the group of mice with residual infection. Bioassay performed to test the infectivity of these cysts produced acute lethal toxoplasmosis following i.p. inoculation in all instances (100%), and importantly, following peroral inoculation in four (29%). The recovered tachyzoites were highly infectious. In addition, significantly elevated interferon gamma (IFN-γ) in the treated mice which developed residual infection compared with any group of infection-free (treated or subinoculated) mice, indicates immunological control of the parasite in the latent form. In conclusion, early treatment of mice infected with *T. gondii* RH tachyzoites with ATO and PDTC induces conversion into tissue cysts, thus providing a new model for studying the mechanism(s) of *T. gondii* stage conversion.

Keywords: *Toxoplasma gondii*; RH strain; Brain cysts; Swiss-Webster mice; Atovaquone; Pyrrolidine dithiocarbamate

1. Introduction

The protozoan *Toxoplasma gondii* is one of the most successful parasites on Earth, capable of infecting a wide range of hosts, including man. It is estimated that up to one quarter of the human population worldwide is infected [1]. *T. gondii* is able to persist in its host by conversion from the proliferative tachyzoite stage into quiescent encysted bradyzoites, controlled in this stage by the host's immune response.

The species consists of a number of morphologically and serologically similar strains, which differ in their virulence in animals [1,2]. The virulence of *T. gondii* strains is commonly assessed according to the outcome of infection in mice. Thus, strains invariably inducing mortality unless mice are treated are considered virulent, and conversely, those inducing chronic infection are considered mouse-avirulent. Studies of the population genetic structure have shown that all *T. gondii* strains belong to three clonal lineages, of which type 1 includes mouse-virulent strains, and types 2 and 3, mouse-avirulent ones [3,4]. A typical type 1 strain is the RH strain, which has ever since its isolation in 1939 [5], been in use in laboratories throughout the world for ease of growth. The RH strain has been reported to have lost its capacity to induce oocyst formation in cats [6], while tissue cysts have been developed in non-immune wild-type mice exclusively after early treatment with sulfadiazine (SDZ) [2,7]. The latter study showed that the RH strain cysts developed in this way have lost their oral infectivity, thus somewhat impeding the use of this procedure as a model for studying tachyzoite/bradyzoite stage conversion as the crucial element in the control of this infection, yet poorly understood.

* Corresponding author. Tel.: +381-11-68-5788; fax: +381-11-64-3691.
E-mail address: olgicadj@imi.bg.ac.yu (O. Djurković-Djaković).

1286-4579/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.micinf.2004.09.016

During experimental evaluation of the therapeutic potential of atovaquone (ATO) combined with pyrrolidine dithiocarbamate (PDTC) in a murine model of acute toxoplasmosis induced with tachyzoites of the RH strain of *T. gondii*, whose results have been preliminarily reported elsewhere [8], the development of tissue cysts was noted as a by-finding. Namely, since the treatment protected a number of mice from death over a period of 6 weeks, the survivors were examined for the presence of residual infection, and some were found to have developed *T. gondii* brain cysts. This naturally arose interest, and we proceeded to assess their virulence, which we report here.

2. Materials and methods

2.1. Mice

Female Swiss-Webster mice (Medical Military Academy Animal Research Facility, Belgrade) weighing 18–20 g at the beginning of each experiment, were used. Mice were housed at six per cage and offered drinking water ad libitum. All animal studies were approved by a local (Institute for Medical Research) ethics committee.

2.2. T. gondii

Tachyzoites of the virulent RH strain maintained through serial intraperitoneal (i.p.) passages were used. For experimental infections, tachyzoites were harvested from mouse peritoneal fluids 72 h postinfection (p.i.) and purified by centrifugation, cotton-wool filtration and needle extraction. The parasites were counted in a hemocytometer, and numbers were adjusted to 2×10^6 per ml with saline. Suspensions were serially 10-fold diluted, and 0.5-ml aliquots of 2×10^2 per ml dilutions were inoculated i.p. into fresh mice.

2.3. Drugs

ATO (micronized powder, lot 291604A, Glaxo-Wellcome, Stevenage, UK) was administered at 5, 25, and 100 mg/kg body mass per day. The doses were chosen on the basis of previous work [9,10], as effective (100 mg/kg per day) or, to better reveal the effects of the combined therapy, as suboptimal (5 and 25 mg/kg per day).

PDTC (ammonium salt, lot 71K2640, Sigma, Steinheim, Germany) was administered at 250 mg/kg per day based on a previous report [11] and our own pilot experiment, which showed that doses up to 300 mg/kg per day do not induce visible toxicity.

Based on the observation that mice consume 4 g of food per day [12], the desired doses were obtained by adding 0.02, 0.1, and 0.4 mg of ATO, respectively, alone or combined with 1 mg of PDTC, per 1 g of ground mouse feed. Fresh food was supplied daily.

2.4. Experimental design

The experimental treatment procedure was described in detail elsewhere [8]. In brief, groups of Swiss-Webster mice (n = 6–12, depending on the experiment) infected i.p. with 10^2 tachyzoites, were randomly assigned to treatment with 5, 25 and 100 mg of ATO/kg per day, alone or combined with 250 mg/kg per day PDTC. Treatment was initiated 24 h p.i. and was continued for 14 days. Two groups of infected mice, one left untreated and one given PDTC alone, served as controls. Survival was monitored and deaths recorded during 6 weeks.

To assess the outcome of the infection in surviving mice, at the end of the 6-week observation period, all survivors were killed by asphyxiation in chloroform, and brains were removed and homogenized for subinoculation into fresh mice to attempt reisolation of *T. gondii* (bioassay). In addition, peripheral blood was collected from the periorbital sinus from both treated and subinoculated survivors for *T. gondii* serology and determination of IFN-γ levels. Since IFN-γ is the major effector molecule in the control of *T. gondii* infection [13], we reasoned that residual infection would be associated with elevated IFN-γ and vice versa, and accordingly, IFN-γ levels would provide further support for the presence/clearance of infection.

2.5. Bioassay

Brains were homogenized in a Teflon homogenizer with 1 ml of saline each and 0.5-ml suspensions were inoculated perorally (p.o.) (by intra-esophageal gavage) into two fresh mice per sample, one each by the i.p. and p.o. routes, or both by the p.o. route. The inoculum sizes were as follows: all brain homogenates in which cysts had been demonstrated were diluted to contain 10 cysts per 0.5 ml, whereas if no brain cysts were demonstrated, the total homogenated brain tissue was inoculated in equal volumes into two mice. Subinoculated mice were monitored daily over a period of 6 weeks; peritoneal fluids of those succumbing were examined for the presence of *T. gondii* tachyzoites. At the end of the 6-week period, all survivors were killed and processed as above for examination of cysts. For cyst enumeration, 25 μl of the brain suspensions was placed on slides and counted under a phase-contrast microscope. The number of cysts per brain was calculated by multiplying the number counted in four drops (by three experienced investigators) by 10, giving a threshold sensitivity of our method of 10 cysts per brain.

2.6. T. gondii serology

T. gondii-specific IgG antibody was detected by the high-sensitivity direct agglutination test (HS-DA), as described by Desmonts and Remington [14], using formalin-fixed RH tachyzoites as antigen, kindly obtained from Philippe Thulliez (Institut de Puériculture, Paris, France). Sera were serially twofold diluted starting from 1:20. The results are expressed as the final positive dilution (serum titer) and converted into international units (IU) according to a WHO reference serum.