Atovaquone maintenance therapy prevents reactivation of toxoplasmic encephalitis in a murine model of reactivated toxoplasmosis

ILDIKO R. DUNAY[1,2], MARKUS M. HEIMESAAT[1], FARIS NADIEM BUSHRAB[3], RAINER H. MÜLLER[3], HARTMUT STOCKER[4], KEIKAWUS ARASTEH[4], MICHAEL KUROWSKI[5], RUDOLF FITZNER[6], KLAUS BORNER[6], and OLIVER LIESENFELD[1,]*

Institute for Infection Medicine, Department of Medical Microbiology and Immunology of Infection, Charité Campus Benjamin Franklin, Hindenburgdamm 27, D-12203 Berlin, Germany[1], Semmelweis University Budapest, Nagyvàrad tèr 4, 1089 Budapest, Hungary[2], Department of Pharmaceutics, Biotechnology and Quality Management, Free University of Berlin, Kelchstr. 31, D-12169 Berlin, Germany[3], Vivantes Auguste-Viktoria Klinikum, Rubensstrasse 125, 12157 Berlin, Germany[4], HIV-Lab, c/o Vivantes Auguste-Viktoria Klinikum, Rubensstrasse 125, 12157 Berlin, Germany[5], Institute of Clinical Chemistry and Pathobiochemistry, Charité Campus Benjamin Franklin, Hindenburgdamm 30, D-12200 Berlin, Germany[6]
* To whom correspondence should be addressed

Running title:

Atovaquone maintenance therapy for reactivated toxoplasmosis

ABSTRACT

Acute therapy with pyrimethamine plus sulfadiazine is the treatment of choice for reactivated

toxoplasmic encephalitis (TE). Acute therapy is followed by lifelong maintenance therapy

(secondary prophylaxis) with the same drugs at lower dosages. The use of pyrimethamine plus

sulfadiazine is hampered by severe side effects including allergic reactions and hematotoxicity.

Alternative treatment regimens with pyrimethamine plus clindamycin or other antiparasitic drugs

are less efficacious. Atovaquone nanosuspensions show excellent therapeutic effects for "acute"

i.v. treatment of reactivated TE in a murine model. In the present study, the therapeutic efficacy

of atovaquone for oral "maintenance" therapy was investigated. Mice with a targeted mutation in

the interferon regulatory factor 8 gene were latently infected with Toxoplasma gondii, developed

reactivated TE, and received acute i.v. therapy with atovaquone nanosuspensions. Mice were then

treated orally with atovaquone suspension or other antiparasitic drugs to prevent relapse of TE.

Maintenance therapy with atovaquone at daily doses of 50 or 100 mg/kg body weight protected

mice against reactivated TE and death. This maintenance treatment was superior to standard

therapy with pyrimethamine plus sulfadiazine. The latter combination was superior to the

combination of pyrimethamine plus clindamycin. Inflammatory changes in the brain parenchyma

and menings as well as parasite numbers in brains of mice confirmed the therapeutic efficacy of

atovaquone for maintenance therapy. Atovaquone was detectable in serum, brains, livers, and

lungs of infected mice by HPLC and/or mass spectrometry. In conclusion, atovaquone appears to

be superior to the standard maintenance therapy regimens in a murine model of reactivated TE.

The therapeutic efficacy of atovaquone for maintenance therapy against TE should be further

investigated in clinical trials.

INTRODUCTION

Toxoplasma gondii (T.g.) is an intracellular protozoan parasite of human and animals with worldwide distribution. Seroprevalence varies with geographical location and up to 70% in Germany and France [1, 2]. Following initial uptake of the parasite in the gut and dissemination throughout the body, the latent stage of infection is characterized by the presence of parasites in cysts in the central nervous system and muscle tissues [2]. Immunocompromised hosts, i.e. patients with AIDS or organ transplant recipients, are at risk of reactivation of the infection by rupture of cysts [2]. Toxoplasmic encephalitis (TE) is the most common clinical manifestation of reactivated disease in AIDS patients who do not receive highly active antiretroviral therapy HAART or antiparasitic prophylaxis TE is the most frequent infectious cause of focal intracerebral lesions in these patients [2-4]. Untreated, reactivation of disease leads to death of the patient. The acute therapy (pyrimethamine plus sulfadiazine) of TE is followed by lifelong maintenance therapy [2, 5]. The standard regimen for maintenance therapy includes pyrimethamine plus sulfadiazine at lower dosages [2]. Pyrimethamine plus sulfadiazine therapy is hampered by severe side effects including hematologic toxicity and/ or life-threatening allergic reactions in between 5 and 15% of patients [5, 6].

The hydroxynaphthoquinone atovaquone is a potent inhibitor of the respiratory chain of parasites with potent in vitro and in vivo activity against both the tachyzoite and cyst forms of *T. gondii* [7-11]. The original formulation of atovaquone (750 mg tablets four times a day) as a single anti-toxoplasmic agent was reported to prevent relapse in 48 of 65 (76%) AIDS patients with mean CD4 counts of 29/μl [12]. More recently, a new formulation of atovaquone (1500 mg suspension) in combination with pyrimethamine or sulfadiazine was reported to prevent relapse in 19 of 20 (95%) patients [11].

We have previously shown the efficacy of atovaquone nanosuspensions in the "acute" i.v. treatment of TE in a murine model [9]. To investigate the therapeutic efficacy of atovaquone for oral "maintenance" therapy, we expanded the murine model of reactivated TE in mice deficient in the interferon regulatory factor 8 (ICSBP/IRF-8) [13] by adding a phase of oral "maintenance" treatment after the course of acute i.v.drug.

Results of the present study reveal that atovaquone maintenance therapy in doses equivalent to the application in humans protected mice against reactivated TE and death. Atovaquone-treated mice did not develop signs of inflammation in the brain parenchyma nor in the meninges. Atovaquone maintenance therapy was superior to standard therapy with pyrimethamine plus sulfadiazine for secondary prophylaxis of TE.

MATERIALS AND METHODS

T. gondii. Cysts of the ME49 strain of *T. gondii* were obtained from brains of NMRI-mice that had been infected intraperitoneally with 10 cysts 2-4 months before. Mice were sacrificed by asphyxiation with CO_2, their brains removed, and triturated in phosphate buffered saline (PBS). An aliquot of the brain suspension was used to determine the numbers of cysts in the preparation by microscopy.

Mice and infection. Inbred female ICSBP/IRF-8$^{-/-}$ mice on C57BL/6-background were bred and maintained under special pathogen-free conditions in the animal facility of the Institute for Infection Medicine, Charité Campus Benjamin Franklin, Berlin. 8-to-12-weeks-old ICSBP/IRF-8$^{-/-}$ mice were orally infected. Mice were treated with sulfadiazine (Sigma-Aldrich, Deisenhofen, Germany) in drinking water (400 mg/l) for 4 weeks beginning 2 days after infection to control latent infection. Two days after discontinuation of sulfadiazine, mice were treated with atovaquone nanosuspensions (10.0 mg/kg body weight) administered as a single i.v.dose on days 2, 5, and 8. At day 9 after discontinuation of sulfadiazine -1 days after discontinuation of acute i.v. atovaquone therapy- daily treatment with antiparasitic drugs as maintenance therapy was initiated p.o. for 1 week. At day 16 – the time point when control mice showed symptoms of disease and/ or began to succumb – their brains, livers, lungs and serum were removed and fixed in formalin for histology or stored at –70°C for high performance liquid chromatography (HPLC) and mass spectrometry (MS) analysis. There were 4 to 6 mice in each experimental group to study mortality and histological changes. HPLC and MS analysis were performed on organs and serum samples of 3 to 4 mice per group. Experiments were repeated at least three times.

Atovaquone. Atovaquone, 2-[trans-4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone was obtained from Glaxo-Smithkline (München, Germany). Atovaquone

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nanosuspensions (ANS) for acute i.v.therapy were produced by high pressure homogenization under aseptic conditions. The drug powder (Glaxo- Smithkline) was dispersed in an aqueous surfactant solution containing of 1% Tween 80 (ICI Surfactants, Eversberg, Belgium), using an Ultra turrax T 25 (Janke and Kunkel, Staufen, D). The coarse pre-dispersion obtained was homogenized in a Micron LAB 40 high pressure homogenizer (APV Systems, Unna, Germany) applying pressures of 150 and 500 bar (2 cycles each), and 1500 bar (20 cycles) [14]. Particles were preserved by thiomersal (Sigma-Aldrich) at a concentration of 0.001% (wt/ vol). Iso-osmolarity was achieved by adjusting with glycerole (Sigma-Aldrich) at a concentration of 2.25% (wt/ vol). Particle size and width of distribution (polydispersity index) were determined by photon correlation spectroscopy (PCS) (Malvern Zetasizer 4, Malvern Instruments, Malvern, U.K.) and laser diffractometry using a Coulter LS 230 (Beckman-Coulter, Krefeld, Germany). Mean diameter measured by PCS was 459 ± 2 nm with a polydispersity index of 0.29 ± 0.02, 99% (vol/ vol) of particles had a diameter of below 2.155 µm (LD99). Atovaquone suspension (Wellvone®, Glaxo-Smithkline) for oral maintenance therapy was diluted in PBS. Mice received 100, 50, or 25mg/kg body weight in a total volume of 0.2 ml by gavage.

Histology. Organs were excised and fixed in a solution containing 5% formalin and 0embedded in paraffin. Sagittal sections of brains and cross sections of livers and lungs were stained with hematoxilin eosin (H&E) according to a standard procedures or by the immunoperoxidase method with rabbit anti-*T. gondii* immunoglobulin G antibody [15]. All reagents for fixing and staining with H&E were obtained from Merck (Darmstadt, Germany). For staining by the immunoperoxidase method, deparaffinized sections were incubated with swine sera at 1:10 (DAKO, Carpinteria, California, USA) and with the primary antibody rabbit-anti-*T. gondii*. For production of rabbit-anti-*T.gondii* antibodies, rabbits were orally infected with 10 cysts ME 49, treated with 300 mg/ l sulfadiazine. Sera were harvested after a boost with *T. gondii*

(RH) 15 days after infection. After rinsing with modified PBS, sections were incubated with swine-anti-rabbit immunglobulin (1:100) (DAKO). Finally sections were incubated with rabbit peroxidase anti-peroxidase (1:100) (Sigma-Aldrich) and with diaminobenzidine (DAKO) development solution after rinsing. Sections stained with H&E were evaluated for inflammatory changes and sections stained with the immunoperoxidase method were evaluated for the numbers of *T. gondii* cysts and *T. gondii* tachyzoites or antigens. Number of inflammatory foci and parasites were counted under 100x magnification in 3 optical fields. A total of 3 sections of each organ of 4 mice in each experimental group were evaluated for the numbers of inflammatory foci and the numbers of *T. gondii* cysts, tachyzoites, and antigens. A score was developed modified after the score described by Araujo et al. [16]. Briefly, normal brain was scored 1. A score of 2 reflected mild meningeal and parenchymal mononuclear cell inflammation, a score of 3 severe meningeal and parenchymal inflammation, and a score of 4 severe meningeal inflammation plus severe parenchymal inflammation with necrosis. The score is given as mean ± SD for at least 4 mice in each group.

High performance liquid chromatography. Weighted tissue samples of serum (200 μl), liver and lung (50-300 mg) were homogenized in 5 ml extraction solution consisting of 2% (vol/ vol) isoamyl alcohol and 98% (vol/ vol) hexane in a glass-teflon homogenizer [17]. 0.1 ml serum was diluted in 5 ml extraction solution. After adding of 1 ml phosphate buffer samples were spun for 20 min in a rotating mixer [18]. Suspensions were centrifuged for 10 min at 2800 g in a temperature controlled centrifuge at 10 °C. Four ml supernatant were evaporated to dryness in a rotating vacuum centrifuge. The dry residue was re-dissolved in mobile phase (aqueous solution of 50% (vol/ vol) acetonitrile and 5% methanol (vol/ vol), pH 2.65) [17-19]. The samples were chromatographed on a reversed-phase column (Spherisorb C1, Waters, USA) guarded with a C 18 precolumn in isocratic mode. Absorbance of the eluate was monitored at 254 nm in a UV

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detector (model LC 95, Perkin Elmer, Überlingen, Germany). The linear calibration function was calculated by means of least squares regression analysis using computer software (SQS 98, Perkin Elmer). Detection limit of this method was 0.6 mg/ l serum. Limits of quantitation for tissues were approximately 0.5 mg/ kg tissue. Inter-assay precision for serum (c.v.) varied from 7.4 to 15.1%. Recovery from spiked serum was 98.1 to 108.1%. Replicate extractions yielded the following extraction rates for the first extraction: 100.0% (serum), 63.6% (brain), 78.1% (liver) and 78.1% (lung).

Mass spectrometry. Since atovaquone concentrations were low in brains of mice treated with atovaquone maintenance therapy, we used mass spectrometry to quantitate atovaquone in brains of mice. 25µl aliquots of serum were mixed with 100µl of internal standard solution containing mycophenolat (Roche, Grenzach, Germany) at a concentration of 0.3 mg/ml. After the addition of 300 µl of acetonitrile (VWR-International, Darmstadt, Germany), samples were vortexed and sonicated for 15 min at room temperature. Samples were then centrifuged at 13000g for 6 min. The supernatants were transferred to clean tubes and aliquots of 20µl were injected onto a reversed-phase C18 column (Eurospher; 5µ; 4,6mm x 30mm; Knauer, Berlin, Germany). The mobile Phase A was distilled water containing 0,0025 M ammonium acetate (VWR-International, Darmstadt, Germany). Mobile Phase B was acetonitrile/ammonium hydroxide, 100/0,008%. The HPLC system consisted of the following components: Rheos 2000 HPLC pump (Flux Instruments[®], Basel, Switzerland) and a 233 XL autosampler (Gilson Abimed[®]). HPLC separation was achieved with mobile phase gradient elution (flow 1,5 ml/min) using the following sequence: 0 min: 100% A; – 0,1 min: 25% A; – 3,0 min: 25% A; - 3,1 min: 100% A; The majority (80%) of the effluent was split off before entering the MS.

An API 3000 mass spectrometer (Applied Biosystems[®],California,USA) equipped with an ESI interface and run with Analyst 1.2 software was used for detection and quantification of

atovaquone in serum and brain samples. Analytes were monitored in the negative MRM mode with the following transitions of precursor to product ions: m/z 365.1 to 171.2 (atovaquone); 318.6 to 275.2 (mycophenolat). The source temperature was set to 400°C.

Standards and quality control samples were prepared in blank mouse serum. For each batch, an 8-point standard calibration curve was analyzed; atovaquone concentrations ranged from 0.617 mg/ml to 79.0 mg/ml.

For quantification of atovaquone in brain tissue the organs were weighed into polypropylene microreaction vials and homogenized mechanically using a pestle. After addition of acetonitrile (5µl/mg brain tissue) samples were vigorously vortexed and sonicated for 60 minutes. The suspended brain tissue was sedimented at 13000 g for 6 minutes and 250 µl aliquots of the supernatant (containing the extract of 50 mg of brain tissue) were transferred into clean polypropylene vials and 20 µl of the internal standard solution (mycophenolat 0,3 mg/ml) added. Chromatographic and MS conditions were as described above. Six calibration standards prepared from blank mouse brain tissue were analyzed with each run. Standard concentrations ranged from 0.051 mg/kg to 3.29 mg/kg.

Statistical analysis. Fisher's exact test was used to compare survival rates. Differences in numbers of inflammatory foci and parasite numbers were analyzed using the Student`s *t*-test.

RESULTS

Determination of optimal time of administration and dosage of atovaquone for acute i.v. treatment of reactivated toxoplasmosis. We have previously reported that mice acutely treated i.v. with atovaquone nanosuspensions (10mg/kg body weight) do not develop reactivated toxoplasmosis whereas all control mice died within 2 weeks after withdrawal of sulfadiazine [9]. To determine the maximum duration between i.v. injections of atovaquone nanosuspensions treatment of mice with 10 mg/kg body weight of atovaquone every other day or every third day was compared (Tab. 1). Both treatment regimens showed equal therapeutic efficacy in the murine model of reactivated toxoplasmosis. Whereas all control mice died, mice treated with 10 mg/kg atovaquone every second or third day survived the infection (Tab. 1). Treated mice did not develop parasite-associated inflammatory changes in their brains (Tab.1). The optimal dose of i.v. atovaquone for treatment of acute reactivated toxoplasmosis was determined by comparing treatment with 10, 5, and 2.5 mg atovaquone nanosuspension/kg body weight. Whereas mice treated with 10 mg atovaquone nanosuspensions did not develop parasite-associated inflammatory changes and survived the infection, 20% and 45% of mice treated with a dose of either 5 or 2.5 mg atovaquone nanosuspensions, respectively developed parasite-associated inflammatory changes in their brains and died. High concentrations of atovaquone were only detectable by HPLC in serum and organs of mice treated with 10 mg/kg body weight (Tab. 1). In contrast, mice treated with 5 mg/kg showed low atovaquone concentrations in serum and liver whereas atovaquone was undetectable by HPLC in brains. Therefore, 10 mg/kg atovaquone nanosuspensions administered every third day, for the i.v. treatment of acute reactivated toxoplasmosis were used in all experiments to investigate the therapeutic efficacy of a variety of antiparasitic drugs for subsequent maintenance therapy.

Murine model of reactivated toxoplasmic encephalitis for the evaluation of maintenance therapy. After modification of the acute treatment phase as described above, we expanded the murine model of acute reactivated toxoplasmosis to include maintenance therapy (Fig. 1). Reactivation of latent toxoplasma infection was induced by withdrawal of sulfadiazine which was used to establish latent infection in the immunocompromized mice [9]. Two days thereafter when mice reactivated the infection, acute therapy with atovaquone nanosuspensions was initiated every third day at a dose of 10 mg/kg body weight (days 2, 5, and 8 after discontinuation of sulfadiazine). One day later, oral maintenance treatment with different antiparasitic drugs was started and administered daily by gavage for 7 days (Fig. 1). 16 days after discontinuation of sulfadiazine, serum and organs were obtained and mortality of mice was monitored in a separate group of mice.

Effect of atovaquone maintenance therapy on survival of mice with reactivated toxoplasmic encephalitis. One day after completion of acute i.v. therapy with atovaquone nanosuspensions, mice were treated with different antiparasitic drugs for 7 days. Control mice began to die within 6 days after discontinuation of acute treatment; all control mice died within 9 days (Fig. 2). In contrast, all mice orally treated with atovaquone suspensions as maintenance therapy (100 mg/kg) survived the infection until the end of the observation period (10 days). The same survival rate was observed in mice treated with 50 mg/kg atovaquone suspension. The combination of pyrimethamine (0.71 mg/kg) plus sulfadiazine (30 mg/kg) administered orally (in doses equivalent to those used in AIDS Patinets) provided partial protection against reactivation; all mice survived the maintenance treatment period of 7 days. Starting on day 8 after initiation of maintenance treatment these mice began to die. The mortality was 14.3% at day 10 after initiation

of maintenance therapy (Fig. 2). The combination of pyrimethamine (0.71 mg/kg) plus clindamycin (35 mg/kg) showed a trend towards inferior efficacy compared to the treatment with atovaquone (p=0.0976). Mice treated with trovafloxacin started to die at 8 days after initiation of maintenance treatment. By day 10, the mortality was 34.0%. Sulfadiazine when administered in drinking water did not protect mice against reactivation of toxoplasmic encephalitis (Fig. 2).

Effect of atovaquone maintenance therapy on histological changes in mice with reactivated toxoplasmic encephalitis. Histological findings in brains and livers obtained on day 7 after initiation of maintenance therapy (time point when maintenance therapy was stopped) paralleled the results of survival as shown above (Tab. 2, Fig. 3). Control mice developed severe meningeal and parenchymal inflammation with numerous parasites and parasite antigens (Fig. 3A); cysts were also present in low numbers (data not shown). In livers of control mice, we detected numerous areas of inflammation associated with parasites (Tab. 2). In contrast, atovaquone maintenance therapy prevented the development of toxoplasmic encephalitis; neither mice treated with 100 mg/kg body weight nor those treated with 50 mg/kg body weight showed any signs of inflammation in their brains (Fig. 3B,C) or livers. Also parasites were undetectable in either organ. Similar results were obtained in mice treated with pyrimethamine plus sulfadiazine (Fig. 3D). However sings of inflammation were observed In contrast, brains of mice treated with pyrimethamine plus clindamycin showed moderate meningeal and parenchymal inflammation (Fig. 3E); low numbers of parasites were detectable primarily in areas of inflammation. A decrease in inflammation both in the meninges and the brain parenchyma was noted in mice treated with trovafloxacin (Fig. 3F).

Atovaquone concentrations in serum and organs. To determine atovaquone concentrations, serum, brains, lungs, and livers were obtained on day 8 after initiation of maintenance therapy. Atovaquone concentrations were determined in serum samples by HPLC and mass spectrometry 24h after the last administration of drug; mice orally treated with 50 or 100 mg/kg atovaquone showed high drug concentrations of 15.00 mg/l or higher in their sera (Fig. 4A). Atovaquone was detectable in livers and lungs at lower concentrations (Fig. 4B). Atovaquone concentrations in brains of mice treated with either 50 or 100 mg/kg were 0.22 ± 0.05 mg/kg and 0.34 ± 0.14 mg/kg, respectively (Fig. 4B).

DISCUSSION

Results of the present study reveal that mice treated orally with atovaquone maintenance therapy in doses equivalent to the application in humans did not develop reactivation TE.

These results were obtained in a new murine model of reactivated toxoplasmosis that closely mimics signs of reactivated toxoplasmosis in immunocompromized patients, including the presence of parasite-associated focal necrotic lesions in the brain parenchyma and meningeal inflammation.This pathological changes resulted in death of mice.

In the past studies on the efficacy of antiparasitic drugs for maintenance therapy have been performed using in vitro systems and/or different animal models [7, 8, 20-22] . However, none of these model systems represented reactivation of latent toxoplasmosis and antiparasitic drugs for maintenance therapy were never administrated orally following a course of acute therapy. Thus, the murine model described in the present study for the first time allowed to adaequately study the efficacy of antiparasitic drugs for maintenance therapy.

The current recommendations for maintenance therapy against TE in patients with AIDS include as first choice the use of sulfadiazine (500 – 1000 mg orally four times daily) plus pyrimethamine (25 – 50 mg orally daily); this recommendation is based on the strong evidence for efficacy and clinical benefit observed in randomized clinical trials (A1 strength of recommendation by the US Public Health Service and the Infectious Disease Society of America) [23]. Alternatively, clindamycin (300 – 450 mg orally every 6 – 8 hours) plus pyrimethamine (25 – 50 mg by mouth daily) or atovaquone (750 mg orally every 6 – 12 hours) with or without

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pyrimethamine (25 – 50 mg orally daily) may be given; the evidence for efficacy of these

alternative treatments is based on clinical experience, descriptive studies, or reports of consulting

committees (CIII strength of recommendation) insufficient to support recommendation [23]. In a

study by Katlama et al. [12], 65 AIDS patients intolerant of standard treatment regimens received

atovaquone (750 mg four times daily) as single maintenance therapy. Atovaquone was found to

be efficacious in 74% of patients [12]. The efficacy of 74% observed in humans by

Katlama and coworkers is lower than the efficacy of 100% observed in mice in the present study.

However, whereas 4 x 750 mg of the old tablet form of atovaquone were administered to patients,

mice received the new suspension formulation of atovaquone in equivocal dosis. It has been

shown, that Hifat (toast with 56g butter) increased the uptake of atovaquone tablets by a factor of

3.9 (AUC) and 5.6 (Cmax) [24]. Atovaquone aqueous suspension or oily solution in miglyol also

increased the AUC and Cmax by a factor of 1.7 and 2.4, respectively[24]. We therefore

hypothesize that the current formulation of atovaquone (oral suspension with Xanthan Gum and

Poloxamer 188, Wellvone® package insert 1997) should prove markedly more efficacious than

the old formulation for maintenance therapy against reactivation of TE in humans. In this respect,

the combination of atovaquone suspension plus either pyrimethamine or sulfadiazine was

effective as maintenance therapy in 19 (95%) of 20 patients with AIDS Chirgwin et al. [11].

However, the study did not allow to determine whether the new atovaquone formulation or rather

the combination therapy mediated the protective effects.

In all regimens including sulfadiazine or pyrimethamine, leucovorin therapy must be

added to prevent bone marrow suppression. In addition, sulfadiazine as well as clindamycin

therapy are hampered by allergic reactions which are observed in approximately 30% of patients;

between 11 and 30% of patients discontinue maintenance therapy [6]. In contrast, atovaquone

maintenance therapy has been reported to be very well tolerated [12]. Only two (3.0%) of 65 AIDS patients experienced gastrointestinal side effects including nausea and vomiting and therefore had to discontinue atovaquone maintenance therapy [6].

We compared the therapeutic effect oral administration of atovaquone in dosages between 25 and 100 mg/kg body weight. Atovaquone dosages of 100 and 50 mg/kg body weight were efficacious for maintenance therapy against TE whereas atovaquone at a dose of 25 mg/kg body weight did not protect mice from reactivated toxoplasmosis (data not shown). Dosage of 50 and 100 mg/kg body weight resulted in serum levels of 14.5 and 20.8 mg/l, respectively; serum levels achieved in infected mice thus fall above the MIC for *T. gondii* reported by us and others to be in the nanogramm range [7-9] The dosage of 50 mg/kg body weight that proved efficacious in the murine model of reactivated TE is equivocal to the dosage reported efficacious in humans (750 mg 4 times daily) by Katlama et al. [12]. Since we were interested in brain concentrations of atovaquone, a mass spectrometry assay for the detection of atovaquone was established. Concentrations achieved in brains of infected mice (0.22 – 0.34 µg/mg) also fell above the MIC of 0,01 µg/ml for *T. gondii*.

In the past, acute therapy for TE has been investigated in murine models using drug-induced immunosuppression [25]. In mice latently infected with the cystogenic ME49 strain, reactivation of TE was induced by a 2-week course of dexamethasone. Administration of atovaquone plus clindamycin (dosages of 50 mg/kg body weight each or higher) significantly prolonged survival of mice and significantly reduced the numbers of brain cysts [25]. A similar effect of atovaquone on cyst numbers was also reported by Araujo et al. [16] in vitro and in a murine model of chronic progressive toxoplasmosis in susceptible CBA/ca mice.

In conclusion, the murine model of reactivated toxopasmosis described in the present study, proved valuable to study efficacy of antiparasitic drugs for maintenance therapy against TE. The therapeutic effect of Atovaquone should be further evaluated a clinical trials.

ACKNOWLEDGMENTS :

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Figure Legends.

FIG. 1. Murine model of reactivated toxoplasmosis in ICSBP/IRF-8^{-/-} mice. After reactivation of latent toxoplasmosis, acute i.v. therapy with atovaquone nanosuspensions was given for 6 days followed by oral maintenance therapy administered for 7 days (see Materials and Methods).

FIG. 2. Survival rate of ICSBP/IRF-8^{-/-} mice treated with atovaquone (50 or 100 mg/kg body weight) or control drugs. After reactivation of latent toxoplasmosis, acute i.v. therapy with atovaquone nanosuspensions was given for 6 days followed by oral maintenance therapy administered for 7 days (see Materials and Methods). At least 5 mice were used in each group. Results shown are pooled from two independent experiments.

FIG. 3. Histological changes in brains of mice with reactivated toxoplasmic encephalitis at day 8 after initiation of maintenance therapy. Small arrows indicate parasitic foci (parasitophorus vacuoles and parasitic antigen), large arrows indicate inflammatory foci. Immunoperoxidase staining, magnification, x100. (A) control mice, atovaquone-treated mice (B) (100mg/kg), (C) (50mg/kg),(D) pyrimethamin plus sulfadiazine-treated mice,(E) pyrimethamin plus clindamicin-treated mice,(F) trovafloxacine-treated mice and (G) sufadiazine-treated mice. Sections shown are representative for at least 4 mice per group; experiments were repeated 3 times.

FIG. 4. Concentrations of atovaquone in serum (A), brains, livers, and lungs (B) of mice with reactivated toxoplasmic encephalitis. Mice were treated with indicated concentrations of atovaquone and killed 7 days after initiation of oral atovaquone maintenance therapy (1 day after thenlast dose of atovaquone). Atovaquone concentrations were determined by mass spectrometry (serum, brains) and HPLC (livers, lungs). Values are derived from at least 3 mice per group and are representative for 2 experiments performed.