Adjunctive bradyzoite-directed therapy for reducing complications of congenital toxoplasmosis

Samuel D. Chorlton*

Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada

ABSTRACT

Congenital toxoplasmosis is caused by *in utero* infection of the fetus with the intracellular parasite *Toxoplasma gondii*. Upon infection, the parasite forms life-long cysts in fetal brain and eyes which are resistant to the currently accepted therapy of pyrimethamine and sulfadiazine. These cysts commonly reactivate later in life causing chorioretinitis and visual impairment, and rarely cause neurological complications. I hypothesize that adjunctive, bradyzoite-directed therapies have the potential to alleviate a significant burden of disease by reducing cyst burden in neonatal brain and eyes. Atovaquone is perhaps the most promising drug for further evaluation given its low side-effect profile, established safety, and efficacy in animal models reducing cyst burden. Very limited observational data in humans suggests atovaquone may prevent *Toxoplasma*-associated chorioretinitis recurrence. Clinical trials are needed to evaluate it and other potential drugs as adjunctive treatment in congenital toxoplasmosis.

Introduction

Congenital toxoplasmosis continues to exact a significant toll on infants around the world, despite complicated prevention and treatment. Between 0.5/1000 and 3.4/1000 live births are affected with the disease depending on geographical region, resulting in 190 100 incident cases per year and 1.20 million DALYs [1]. Clinical presentation of the disease ranges from asymptomatic to severe neurological impairment and death [2]. At birth, a majority of infected neonates are asymptomatic; affected neonates can present with chorioretinitis, hydrocephalus, seizures and intracranial calcifications [2]. Both asymptomatic and symptomatic treated infants are at risk for late ophthalmologic and neurodevelopmental complications. In a prospective study of 477 infants twith congenital toxoplasmosis, treated with standard therapy and followed for a median of 10.5 years, the cumulative probability of new retinal lesions was 48% by 20 years of age [3]. The WHO has estimated that chorioretinitis occurs in 39% of North American and European cases of congenital toxoplasmosis, and 90% of South American cases, while other studies have shown rates as low as 26% [1,4]. In about 1–2% of cases, treated infants also go on to develop neurodevelopmental complications, including developmental delay, seizures and hydrocephalus [3–5].

Congenital toxoplasmosis is caused by vertical infection of the fetus with the intracellular parasite *Toxoplasma gondii*. Transmission almost always occurs during primary infection of the mother via exposure to contaminated soil, water, or undercooked meat [6]. The time of maternal infection is correlated with the risk of vertical transmission (15%

at 13 weeks gestational age vs. 71% at 36 weeks) and inversely correlated with severity of complications [7]. In acute infection, *T. gondii* tissue cysts, containing the bradyzoite form of the parasite, or oocysts, containing the sporozoite form of the parasite, convert into tachyzoites in maternal gastrointestinal epithelium and enter her blood. These tachyzoites are fast replicating and cross the placenta to invade fetal neural tissues. Starting as early as 7 days after infection of the fetal brain, tachyzoites convert back into bradyzoites and form life-long tissue cysts within the brain and eyes [8].

Postnatal treatment guidelines for congenital toxoplasmosis suggest a combination of pyrimethamine, sulfadiazine or sulfadoxine, and folinic acid for one year [2]. This recommendation is based on expert opinion and observational studies showing a lower incidence of long term complications compared with shorter treatment in historical controls, although there are also significant risks of treatment including neutropenia, thrombocytopenia, megaloblastic anemia, allergic reaction and renal failure [9,10]. More importantly, however, is the lack of activity of these drugs against the bradyzoite stage of *T. gondii*. Periodically and for unknown reasons, bradyzoites reactivate causing overt disease and the aforementioned late complications of congenital toxoplasmosis [11,12]. In fact, pyrimethamine and sulfadiazine therapy have enhanced conversion of tachyzoites into bradyzoites in some animal models, possibly by increasing cellular stress [13–15].

The hypothesis

Given the high rate of disease recurrence caused by bradyzoite

reactivation in infants treated for congenital toxoplasmosis, bradyzoite-directed therapy, in addition to standard postnatal therapy, has the potential to reduce a significant burden of late complications.

Evaluation of the hypothesis

There is no evidence, to my knowledge, directly looking at bradyzoite-directed therapy and disease recurrence. However, several lines of evidence support the hypothesis that reducing cyst burden, even if not eliminating it, would reduce risk for disease recurrence in congenital infection. First, in humans with *Toxoplasma* encephalitis, a greater number of patients with recurrence of encephalitis, compared with first episode encephalitis, have bradyzoite specific markers detected in their CSF, suggesting that bradyzoite presence is specifically associated with relapse [16,17]. Secondly, a greater proportion of mice inoculated with a higher number of cysts, either via ingestion or subcutaneous injection, develop acute toxoplasmosis compared with mice inoculated with a lower number. Mice inoculated with higher burdens of cysts also have more severe disease when they do develop it [18–20]. Finally, in a model of chronically infected mice undergoing immunosuppression, mice that developed *Toxoplasma* encephalitis had a 6-fold increase in the number of brain cysts compared with mice that underwent the same immunosuppression but did not develop encephalitis [21]. Taken together, these findings show that cyst presence and number are likely causal factors in predicting toxoplasmosis recurrence.

Two recent reviews highlight currently approved and investigational drugs with activity against the bradyzoite stage of *Toxoplasma gondii* and the potential to decrease cyst burden [22,23]. Notably, animal studies have demonstrated activity of spiramycin combined with metronidazole, itraconazole, atovaquone (with or without clindamycin), guanabenz, didanosine, and miltefosine against chronic *Toxoplasma* infection *in vivo* [24–35]. All of these agents are approved for use in humans, and some of these agents are established and safe in pediatrics. In this section, I review support for what I consider to be some of the most promising therapeutic research hypotheses.

Atovaquone

Atovaquone is perhaps the most promising adjunctive agent for further evaluation given its low side effect profile, selective antiparasitic activity, single drug administration, lack of interaction with standard congenital toxoplasmosis therapy, and FDA approval for other pediatric disease (ie malaria with proguanil) [36,37].

In animal studies of chronic *Toxoplasma* infection, atovaquone has been shown to reproducibly and reliably reduce the number of brain cysts [28,29,31,32,34,35]. Compared with pyrimethamine and sulfadiazine, atovaquone was significantly superior in reducing the number of brain cysts in models of both acute and chronic toxoplasmosis [31]. In the acute model, where mice were treated upon the first sign of retinal lesions for a total of 4 weeks, atovaquone-treated mice had a mean of 1.5 (SEM 0.52) cysts per whole brain. This number was compared with control-treated or pyrimethamine- and sulfdiazine-treated mice, which had a mean of 12.89 (SEM 2.91) and 8.4 (SEM 1.93) cysts per brain, respectively ($p < 0.05$). In the chronic model, where treatment was started 12 weeks after *Toxoplasma* inocculation of the mice, atovaquone-treated mice had 17.4 cysts per brain, compared with 318 in the control group and an unspecified but similar (to the control) number in the pyrimethamine- and sulfadiazine-treated group. Numbers of cysts in animal eyes in this trial were too small for meaningful between-group analysis. Some evidence also suggests that the addition of clindamycin to atovaquone synergistically enhances clearance of brain cysts in mice [32]. Whether this effect is specific to clindamycin, or applies to atovaquone combined with sulfadiazine and pyrimethamine is unknown. Clindamycin has already been recommended and used for congenital toxoplasmosis in children with sulfadiazine allergy or G6PD deficiency, but as coexistence of these conditions is

rare, it is infrequently used [38].

In humans, atovaquone has been successfully used for acute toxoplasmosis in adults, either in combination with pyrimethamine and folinic acid, or with sulfadiazine, or as a single agent [39–41]. In two uncontrolled trials, one prospective and one retrospective, of chorioretinitis treated with atovaquone, there appeared to be a lower recurrence rate of eye lesions than in historical benchmarks using other antiparasitics [42,43]. Winterhalter et al. show that at 2 years, 27% of their 41 patients had disease recurrence, compared with 35% and 53% in select other studies [42,44,45]. Pearson et al. found that in 16 patients completing treatment for chorioretinis, only 1 developed disease recurrence during an average follow up of 10 months [43]. These studies, however, are severely limited by their observational nature and lack of direct comparison.

In other studies of acute toxoplasmosis in humans, atovaquone has had high rates of failure and relapse [40,41,46,47]. Through these early trials and animal studies, it was realized that atovaquone should not be used alone and was synergistic when used in combination with other anti-*Toxoplasma* therapies [30,48]. The ACTG 237/ANRS 039 study showed that atovaquone used in combination with pyrimethamine or sulfadizaine had comparable response and relapse rates to standard combination therapy in AIDS patients with *Toxoplasma* encephalitis. No trials have evaluated atovaquone in addition to two-drug standard therapy, either for acute outcomes or relapse rates.

The last disadvantage of atovaquone is its cost. In 2007, atovaquone was noted to cost €116 per week in comparison to pyrimethamine (€6.50 per week), sulfadiazine (€25 per week) and clindamycin (€30 per week) [49]. However, given the large burden of disease caued by recurrent toxoplasmosis, some have postulated that atovaquone therapy would be cost-saving [42].

Other potential agents

Spiramycin has historically been used in some centers for postnatal treatment of congenital toxoplasmosis; however, since 2001 it has been phased out given limited CNS penetration and parasitostatic action [2]. Chew et al. showed that spiramycin, coadministered with metronidazole, reduced the number of brain cysts 15-fold compared with placebo in mice after just one week of therapy [24]. In this model, metronidazole functioned by increasing brain spiramycin $AUC_{0-\infty}$ by 64%, likely by inhibition of multidrug-resistant protein 2 and P-glycoprotein. Metronidazole is commonly used for neonatal anaerobic infections, and is generally well tolerated. There are no interactions of this combination with standard therapy. One potential drawback is that metronidazole lowers the seizure threshold, and neonates with congenital toxoplasmosis are predisposed to seizures, possibly limiting the utility of this combination.

The antifungal itraconazole has also been shown to reduce brain cysts in an animal model [27]. Interestingly, itraconazole achieves negligible CSF concentrations but high brain parenchymal concentrations [50]. Haynes et al. found that brain parenchymal concentrations of hydroxyl-itraconazole, the active metabolite of itraconazole, approached those measured in serum of mice with *Histoplasma* meningitis [51]. In efficacy studies, itraconazole has had comparable efficacy with fluconazole in an animal model of coccidioidal meningitis and in some, but not all, human trials of consolidation therapy in cryptococcal meningitis [52–54]. Furthermore, despite significant interactions between azoles and other drugs, itraconazole does not appear to interact with standard congenital toxoplasmosis therapy. Given highly variable drug absorption, itraconazole usually requires serum drug level monitoring. Itraconazole can also cause hepatic dysfunction, hypokalemia, hypertension, and peripheral edema, necessitating routine laboratory monitoring. As standard of care for congenital toxoplasmosis usually incorporates frequent bloodwork and followup of therapy toxicity, this is unlikely to be a barrier to administration.

While these drugs have been shown to reduce cyst burden in animal

models, none have been replicated in additional studies, and none have been compared against pyrimethamine and sulfadiazine. Further research is needed to confirm their efficacy in animal models before consideration of trials in humans.

Unlikely candidates

Miltefosine, guanabenz and didanosine are also drugs approved for use in humans and have been shown to reduce cyst burden, but are unlikely to be tested in congenital toxoplasmosis any time soon [25,26]. Miltefosine is a repurposed cancer drug, currently used for leishmaniasis, and exhibits embryotoxicity and teratogenicity. Safety in children under 12 years of age has not been established, and rat studies show juveniles to be more sensitive to adverse effects including retinal degenration and renal toxicity [55]. Similarly, there is no clinical experience for guanabenz, a retired antihypertensive, in pediatrics. Finally, didanosine has been used in pediatric HIV and exhibits significant toxicities, including a potentially fatal hepatotoxicity, and has therefore been removed from HIV guidelines [56].

Consequences of the hypothesis and discussion

At the time of writing (July 2019), there are five and two trials registered in ClinicalTrials.gov and the WHO's International Clinical Trials Registry Platform, respectively, with keywords 'congenital toxoplasmosis'; none of these trials examine novel treatment strategies directed at the *T. gondii* bradyzoite. The agents used against toxoplasmosis have remained largely unchanged since the 1950s despite continued poor outcomes for many patients [57]. Collectively, atovaquone and the other listed drugs in this hypothesis represent a hypothetical path forward in the treatment of congenital toxoplasmosis; however, there is currently not enough evidence to support any of them for clinical use. Researchers and funders should prioritize evaluation of adjunctive agents against the *Toxoplasma* bradyzoite with the hope of reducing long-term complications of congenital toxoplasmosis.

Financial support

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.109376.

References

[1] Torgerson PR, Mastroiacovo P. The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ 2013;91:501–8. https://doi.org/10.2471/BLT.12.111732.

[2] Maldonado YA, Read JS. Diseases C on I. Diagnosis, treatment, and prevention of congenital toxoplasmosis in the United States. Pediatrics 2017;139:e20163860 https://doi.org/10.1542/peds.2016-3860.

[3] Wallon M, Garweg JG, Abrahamowicz M, Cornu C, Vinault S, Quantin C, et al. Ophthalmic outcomes of congenital toxoplasmosis followed until adolescence. Pediatrics 2014;133:e601–8. https://doi.org/10.1542/peds.2013-2153.

[4] Berrébi A, Assouline C, Bessières M-H, Lathière M, Cassaing S, Minville V, et al. Long-term outcome of children with congenital toxoplasmosis. Am J Obstet Gynecol 2010;203:552.e1–6. https://doi.org/10.1016/j.ajog.2010.06.002.

[5] Peyron F, Wallon M, Bernardoux C. Long-term follow-up of patients with congenital ocular toxoplasmosis. N Engl J Med 1996;334:993–4. https://doi.org/10.1056/NEJM199604113341517.

[6] Cook AJC, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, et al. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. BMJ 2000;321:142–7.

[7] SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group, Thiébaut R, Leproust S, Chêne G, Gilbert R. Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. Lancet Lond Engl 2007;369:115–22. https://doi.org/10.1016/S0140-6736(07)60072-5.

[8] Dubey JP, Lindsay DS, Speer CA. Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin Microbiol Rev 1998;11:267–99.

[9] Roizen N, Swisher CN, Stein MA, Hopkins J, Boyer KM, Holfels E, et al. Neurologic and developmental outcome in treated congenital toxoplasmosis. Pediatrics 1995;95:11–20.

[10] McLeod R, Boyer K, Karrison T, Kasza K, Swisher C, Roizen N, et al. Outcome of treatment for congenital toxoplasmosis, 1981–2004: the national collaborative chicago-based, congenital toxoplasmosis study. Clin Infect Dis 2006;42:1383–94. https://doi.org/10.1086/501360.

[11] Bhopale GM. Pathogenesis of toxoplasmosis. Comp Immunol Microbiol Infect Dis 2003;26:213–22. https://doi.org/10.1016/S0147-9571(02)00058-9.

[12] Roberts F, McLeod R. Pathogenesis of toxoplasmic retinochoroiditis. Parasitol Today 1999;15:51–7. https://doi.org/10.1016/S0169-4758(98)01377-5.

[13] Costa IN, Angeloni MB, Santana LA, Barbosa BF, Silva MCP, Rodrigues AA, et al. Azithromycin Inhibits Vertical Transmission of Toxoplasma gondii in Calomys callosus (Rodentia: Cricetidae). Placenta 2009;30:884–90. https://doi.org/10.1016/j.placenta.2009.08.002.

[14] Sullivan WJ, Hakimi M-A. Histone mediated gene activation in Toxoplasma gondii. Mol Biochem Parasitol 2006;148:109–16. https://doi.org/10.1016/j.molbiopara.2006.03.010.

[15] Belal US, Norose K, Aosai F, Mun H-S, Ahmed AK, Chen M, et al. Evaluation of the effects of sulfamethoxazole on Toxoplasma gondii loads and stage conversion in IFN-γ knockout mice using QC-PCR. Microbiol Immunol 2004;48:185–93. https://doi.org/10.1111/j.1348-0421.2004.tb03504.x.

[16] Contini C, Cultrera R, Seraceni S, Segala D, Romani R, Fainardi E, et al. The role of stage-specific oligonucleotide primers in providing effective laboratory support for the molecular diagnosis of reactivated Toxoplasma gondii encephalitis in patients with AIDS. J Med Microbiol 2002;51:879–90. https://doi.org/10.1099/0022-1317-51-10-879.

[17] Cultrera R, Seraceni S, Contini C. Efficacy of a novel reverse transcriptase-polymerase chain reaction (RT-PCR) for detecting Toxoplasma gondii bradyzoite gene expression in human clinical specimens. Mol Cell Probes 2002;16:31–9. https://doi.org/10.1006/mcpr.2001.0394.

[18] da Silva RC, da Silva AV, Langoni H. Recrudescence of Toxoplasma gondii infection in chronically infected rats (Rattus norvegicus). Exp Parasitol 2010;125:409–12. https://doi.org/10.1016/j.exppara.2010.04.003.

[19] Freyre A, Falcón J, Mendez J, Gonzalez M. Toxoplasma gondii: Protection against colonization of the brain and muscles in a rat model. Exp Parasitol 2008;119:252–5. https://doi.org/10.1016/j.exppara.2008.02.007.

[20] Freyre A, Falcón J, Méndez J, González M. Toxoplasma gondii: an improved rat model of congenital infection. Exp Parasitol 2008;120:142–6. https://doi.org/10.1016/j.exppara.2008.06.007.

[21] Djurkovic Djakovic O, Milenkovic V. Murine model of drug-induced reactivation of Toxoplasma gondii. Acta Protozool 2001;40:99–106.

[22] Neville AJ, Zach SJ, Wang X, Larson JJ, Judge AK, Davis LA, et al. Clinically available medicines demonstrating anti-toxoplasma activity. Antimicrob Agents Chemother 2015;59:7161–9. https://doi.org/10.1128/AAC.02009-15.

[23] Montazeri M, Mehrzadi S, Sharif M, Sarvi S, Shahdin S, Daryani A. Activities of anti-Toxoplasma drugs and compounds against tissue cysts in the last three decades (1987 to 2017), a systematic review. Parasitol Res 2018;117:3045–57. https://doi.org/10.1007/s00436-018-6027-z.

[24] Chew WK, Segarra I, Ambu S, Mak JW. Significant reduction of brain cysts caused by Toxoplasma gondii after treatment with spiramycin coadministered with metronidazole in a mouse model of chronic toxoplasmosis. Antimicrob Agents Chemother 2012;56:1762–8. https://doi.org/10.1128/AAC.05183-11.

[25] Sarciron ME, Lawton P, Saccharin C, Petavy AF, Peyron F. Effects of 2',3'-dideoxyinosine on Toxoplasma gondii cysts in mice. Antimicrob Agents Chemother 1997;41:1531–6.

[26] Eissa MM, Barakat AMA, Amer EI, Younis LK. Could miltefosine be used as a therapy for toxoplasmosis? Exp Parasitol 2015;157:12–22. https://doi.org/10.1016/j.exppara.2015.06.005.

[27] Martins-Duarte ES, Lemgruber L, de Souza W, Vommaro RC. Toxoplasma gondii: fluconazole and itraconazole activity against toxoplasmosis in a murine model. Exp Parasitol 2010;124:466–9. https://doi.org/10.1016/j.exppara.2009.12.011.

[28] Araujo FG, Huskinson-Mark J, Gutteridge WE, Remington JS. In vitro and in vivo activities of the hydroxynaphthoquinone 566C80 against the cyst form of Toxoplasma gondii. Antimicrob Agents Chemother 1992;36:326–30.

[29] Ferguson DJ, Huskinson-Mark J, Araujo FG, Remington JS. An ultrastructural study of the effect of treatment with atovaquone in brains of mice chronically infected with the ME49 strain of Toxoplasma gondii. Int J Exp Pathol 1994;75:111–6.

[30] Araujo FG, Lin T, Remington JS. The activity of atovaquone (566C80) in murine toxoplasmosis is markedly augmented when used in combination with pyrimethamine or sulfadiazine. J Infect Dis 1993;167:494–7. https://doi.org/10.1093/infdis/167.2.494.

[31] Gormley PD, Pavesio CE, Minnasian D, Lightman S. Effects of drug therapy on Toxoplasma cysts in an animal model of acute and chronic disease. Invest Ophthalmol Vis Sci 1998;39:1171–5.

[32] Djurković-Djaković O, Milenković V, Nikolić A, Bobić B, Grujić J. Efficacy of ato-vaquone combined with clindamycin against murine infection with a cystogenic (Me49) strain of Toxoplasma gondii. J Antimicrob Chemother 2002;50:981–7. https://doi.org/10.1093/jac/dkf251.

[33] Benmerzouga I, Checkley LA, Ferdig MT, Arrizabalaga G, Wek RC, Sullivan WJ. Guanabenz repurposed as an antiparasitic with activity against acute and latent Toxoplasmosis. Antimicrob Agents Chemother 2015;59:6939–45. https://doi.org/10.1128/AAC.01683-15.

[34] Sordet F, Aumjaud Y, Fessi H, Derouin F. Assessment of the activity of atovaquone-loaded nanocapsules in the treatment of acute and chronic murine toxoplasmosis. Parasite 1998;5:223–9. https://doi.org/10.1051/parasite/1998053223.

[35] Araujo FG, Huskinson J, Remington JS. Remarkable in vitro and in vivo activities of the hydroxynaphthoquinone 566C80 against tachyzoites and tissue cysts of Toxoplasma gondii. Antimicrob Agents Chemother 1991;35:293–9.

[36] Hudson AT. Atovaquone—a novel broad-spectrum anti-infective drug. Parasitol Today 1993;9:66–8. https://doi.org/10.1016/0169-4758(93)90040-M.

[37] U.S. Food & Drug Administration. SUPPL-23: labeling-package insert for MALARONE; 2019.

[38] Nunes AA. Patient with toxoplasmosis and glucose-6-phosphate dehydrogenase deficiency: a case report. Cases J 2009;2. https://doi.org/10.4076/1757-1626-2-8826.

[39] Chirgwin K, Hafner R, Leport C, Remington J, Andersen J, Bosler EM, et al. Randomized phase II trial of atovaquone with pyrimethamine or sulfadiazine for treatment of Toxoplasmic encephalitis in patients with acquired immunodeficiency syndrome: ACTG 237/ANRS 039 study. Clin Infect Dis 2002;34:1243–50. https://doi.org/10.1086/339551.

[40] Kovacs JA. Efficacy of atovaquone in treatment of toxoplasmosis in patients with AIDS. The NIAID-Clinical Center Intramural AIDS Program. Lancet Lond Engl 1992;340:637–8. https://doi.org/10.1016/0140-6736(92)92172-c.

[41] Torres RA, Weinberg W, Stansell J, Leoung G, Kovacs J, Rogers M, et al. Atovaquone for salvage treatment and suppression of toxoplasmic encephalitis in patients with AIDS. Clin Infect Dis Off Publ Infect Dis Soc Am 1997;24:422–9. https://doi.org/10.1093/clinids/24.3.422.

[42] Winterhalter S, Severing K, Stammen J, Maier AK, Godehardt E, Joussen AM. Does atovaquone prolong the disease-free interval of toxoplasmic retinochoroiditis? Graefes Arch Clin Exp Ophthalmol 2010;248:1187–92. https://doi.org/10.1007/s00417-010-1379-9.

[43] Pearson PA, Piracha AR, Sen HA, Jaffe GJ. Atovaquone for the treatment of tox-oplasma retinochoroiditis in immunocompetent patients11The authors have no fi-nancial interest in atovaquone or Burroughs Wellcome Co. Ophthalmology 1999;106:148–53. https://doi.org/10.1016/S0161-6420(99)90021-0.

[44] Tugal-Tutkun I, Corum I, Otük B, Urgancioglu M. Active ocular toxoplasmosis in Turkish patients: a report on 109 cases. Int Ophthalmol 2005;26:221–8. https://doi.org/10.1007/s10792-007-9047-8.

[45] Garweg JG, Scherrer JN, Halberstadt M. Recurrence characteristics in European patients with ocular toxoplasmosis. Br J Ophthalmol 2008;92:1253–6. https://doi.org/10.1136/bjo.2007.123661.

[46] Katlama C, Mouthon B, Gourdon D, Lapierre D, Rousseau F. Atovaquone as long-term suppressive therapy for toxoplasmic encephalitis in patients with AIDS and multiple drug intolerance. Atovaquone Expanded Access Group. AIDS Lond Engl 1996;10:1107–12.

[47] Baatz H, Mirshahi A, Puchta J, Gümbel H, Hattenbach L-O. Reactivation of Toxoplasma retinochoroiditis under atovaquone therapy in an immunocompetent patient. Ocul Immunol Inflamm 2006;14:185–7. https://doi.org/10.1080/09273940600659740.

[48] Romand S, Pudney M, Derouin F. In vitro and in vivo activities of the hydro-xynaphthoquinone atovaquone alone or combined with pyrimethamine, sulfadia-zine, clarithromycin, or minocycline against Toxoplasma gondii. Antimicrob Agents Chemother 1993;37:2371–8. https://doi.org/10.1128/AAC.37.11.2371.

[49] Pleyer U, Torun N, Liesenfeld O. Okuläre Toxoplasmose. Ophthalmology 2007;104:603–16. https://doi.org/10.1007/s00347-007-1535-8.

[50] Kethireddy S, Andes D. CNS pharmacokinetics of antifungal agents. Expert Opin Drug Metab Toxicol 2007;3:573–81. https://doi.org/10.1517/17425225.3.4.573.

[51] Haynes RR, Connolly PA, Durkin MM, LeMonte AM, Smedema ML, Brizendine E, et al. Antifungal therapy for central nervous system histoplasmosis, using a newly developed intracranial model of infection. J Infect Dis 2002;185:1830–2. https://doi.org/10.1086/340825.

[52] Sorensen KN, Sobel RA, Clemons KV, Pappagianis D, Stevens DA, Williams PL. Comparison of fluconazole and itraconazole in a rabbit model of coccidioidal me-ningitis. Antimicrob Agents Chemother 2000;44:1512–7.

[53] van der Horst CM, Saag MS, Cloud GA, Hamill RJ, Graybill JR, Sobel JD, et al. Treatment of cryptococcal meningitis associated with the acquired im-munodeficiency syndrome. N Engl J Med 1997;337:15–21. https://doi.org/10.1056/NEJM199707033370103.

[54] Saag MS, Cloud GA, Graybill JR, Sobel JD, Tuazon CU, Johnson PC, et al. A Comparison of itraconazole versus fluconazole as maintenance therapy for AIDS-associated cryptococcal meningitis. Clin Infect Dis 1999;28:291–6. https://doi.org/10.1086/515110.

[55] U.S. Food & Drug Administration. Miltefosine label; 2014.

[56] Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the use of antiretroviral agents in pediatric HIV infection: archived drugs; 2019.

[57] Weiss LM, Dubey Jitender P. Toxoplasmosis: a history of clinical observations. Int J Parasitol 2009;39:895–901. https://doi.org/10.1016/j.ijpara.2009.02.004.